UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

**OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES**

MEMORANDUM

October 5, 2006
TXR # 0050504

SUBJECT: Flufenacet: Review of Developmental Neurotoxicity Study

PC Code: 121903
DP Barcode: D274207, D288567

FROM: Kathleen C. Raffaele, Ph.D.   *Kathleen C. Raffaele*   *10/5/06*
Toxicology Branch
Health Effects Division (7509P)

TO: Jack Arthur
Health Effects Division (7505P)

THROUGH: Alberto Protzel, P.h.D.   *[signature]*   10/5/06
Branch Senior Scientist
Toxicology Branch
Health Effects Division (7509P)

I.   CONCLUSIONS

The submitted developmental neurotoxicity study (MRID 45232501, MRID 45796117), reviewed by Susan Makris, is attached. It has been classified Acceptable/Nonguideline. Additional data were requested, as described (diet concentration analyses, missing morphometric histopathology data, procedural information for functional observation assessments, additional data for learning and memory evaluations, and adequate positive control data). The DER is attached.

II.   ACTION REQUESTED

Review submitted Developmental Neurotoxicity study for Flufenacet (MRID 45232501, 45796117)

DATA EVALUATION RECORD

FLUFENACET/121903

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 45232501

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 02-29

Primary Reviewer:
Carol S. Forsyth, Ph.D., D.A.B.T

Signature:
Date: MAY 1 3 2002

Secondary Reviewers:
Cheryl B. Bast, Ph.D., D.A.B.T.

Signature:
Date: MAY 1 3 2002

Robert H. Ross, M.S. Group Leader

Signature:
Date: MAY 1 3 2002

Quality Assurance:
Susan S. Chang, M.S.

Signature:
Date: MAY 1 3 2002

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

EPA Reviewer: Susan L. Makris, M.S.   Signature: _Susan S Makris_
Toxicology Branch, Health Effects Division (7509C)   Date _3/6/04_
EPA Work Assignment Manager: PV Shah, Ph.D.   Signature: _P VShah_
Registration Action Branch 1, Health Effects Division (7509C)   Date _3 | 16 | 04_

TXR#: 0050504

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 121903   **DP BARCODE:** D274207, D288567
                                        SUBMISSION NO.: S595659, S628843

TEST MATERIAL (PURITY): Flufenacet (96.9% a.i.)

SYNONYMS: Thiafluamide; BAY FOE 5043 Technical; N-(4-fluorophenyl)-N-(1-methylethyl)-2-([5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxyl) acetamide

CITATION: Hoberman, A.M. (2000) Developmental neurotoxicity study of technical grade flufenacet administered orally via diet to Crl:CD®BR VAF/Plus® presumed pregnant rats. Argus Research Laboratories, Inc., Horsham, PA. Study No. 98-C472-SE, Bayer Report No. 109810, September 12, 2000. MRID 45232501. Unpublished

Sheets, L. (2001) Supplemental submission to Bayer Report No. 109810: Developmental neurotoxicity study of technical grade flufenacet administered orally via diet to Crl:CD® BR VAF/Plus® rats (Argus Research Laboratories, Inc., Horsham, PA, Study No. 98-C472-SE, Bayer report no. 109810-1, September 12, 2000). June 27, 2001. MRID 45796117. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, Box 4913, Hawthorn Road, Kansas City, MO 64120

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45232501), flufenacet (96.9% a.i., batch #603-0013) was administered to 25 pregnant female Crl:CD®BR VAF/Plus® rats/group at dietary concentrations of 0, 20, 100, or 500 ppm from gestation day 6 through postnatal day 10. Doses for the treated groups were 1.7, 8.3, and 40.8 mg/kg/day, respectively, during gestation and 3.0, 15.4, and 76.7 mg/kg/day, respectively, during lactation days 1-12. Body weight and food consumption data were recorded for dams. Detailed clinical observations, including assessments of autonomic function, were conducted daily during gestation and on LD 1, 5, 8, 14, and 22. Dams were killed and necropsied on LD 22. Offspring body weights, developmental landmarks (surface righting, pinna unfolding, eye opening, acoustic startle response, and pupil constriction), and survival were assessed. On PND 5, litters were

standardized to yield 5 males and 5 females (as closely as possible). On PND 12, pups were randomly assigned to each of the following five subsets: 1) fixed brain weights and/or neuropathological evaluation on PND 12 (6/sex/group); 2) passive avoidance testing (on PND 23-25 and 30-32) and water maze testing (on PND 59-62 and 66-69) (20/sex/group); 3) motor activity testing (on PND 14, 18, 22, and 60) and auditory startle habituation (on PND 23 and 61) (20/sex/group); 4) fixed brain weights and neuropathological evaluation on PND 74-77 (6/sex/group); and 5) replacement pups. In addition, the pups from subsets 1 and 5 were used for evaluation of liver and fixed thyroid/parathyroid weights, and assessment of thyroid hormone levels (T_3 and T_4) on PND 12 and 22, and the pups from subsets 2-4 were observed for the age of attainment of balanopreputial separation or vaginal patency.

All dams survived to scheduled termination and no clinical signs of toxicity were observed. During gestation, transient decreases in maternal body weight, body weight gain, and food consumption were observed in the mid- and high-dose groups (100 and 500 ppm) following the initiation of dietary treatment. Absolute body weights for the mid- and high-dose groups were significantly less than those of the control group on gestation days (GD) 8-9 (96% of control) and GD 8-12 (93-96% of control), respectively. Significant reductions in body weight gains were observed for the mid-dose group during GD 6-9 (47% of control) and for the high-dose group for the intervals of GD 6-9 (37% of control) and 6-21 (86% of control). Decreases in body weight gains correlated with significantly reduced food consumption as compared to controls during GD 6-9 for the mid-dose group (84% of control) and GD 6-12 for the high-dose group (72-84% of control). Relative food consumption was also significantly decreased for the mid-dose group (GD 6-9) and the high-dose group (GD 6-12). There were no treatment-related effects on maternal body weight or food consumption during the lactation period. Reproductive performance was not affected by treatment. In light of their small magnitude, short duration, and correlation with decreased food consumption (indicative of a possible palatability problem), the decreases in maternal body weight and body weight gain during early gestation were not considered adverse. **The maternal LOAEL for flufenacet in rats is not determined. The maternal NOAEL is 500 ppm (40.8 mg/kg/day).**

There were no effects of treatment on offspring survival. Offspring development and growth were affected in all treated groups. Significant decreases in body weight and body weight gain were observed. These weight deficits were observed starting at PND 5 for high-dose pups, and at PND 12 for low- and mid-dose pups. For the period of PND 5-22, body weight gains were 87-89% of control at 20 ppm, 82-84% of control at 100 ppm, and 86-88% of control at 500 ppm. Postweaning recovery to control body weight levels was observed in low-dose offspring and in mid-dose females. Other evidence of delayed offspring development at the mid- and high doses included significant delays in the age at eye opening and in the age of preputial separation in males.

Serum T_3 and T_4 measures of blood collected at PND 12 and 22 were not affected by treatment. No treatment-related effects on liver or thyroid/parathyroid weights or histopathology were observed in the offspring.

Neurobehavioral assessment of the offspring revealed no treatment-related effects on autonomic function, auditory startle habituation, or learning and memory testing (passive avoidance and

water maze). Treatment-related decreases in motor activity counts were observed in PND 14 female pups at the mid- and high-dose. Neuropathological evaluation revealed significant decreases (9%) in caudate putamen measurements for the adult female offspring at the high dose as compared to control. Subsequent analysis of the mid-dose brains confirmed a similar significant response (5-10% decreases from control values); low-dose brains were not evaluated.

The offspring LOAEL is 20 ppm (1.7 mg/kg/day), based on decreased preweaning body weight and body weight gain. The offspring NOAEL is not determined. In addition to the decreased body weight and body weight gain observed at the LOAEL, significant treatment-related findings at 100 ppm (8.3 mg/kg/day) and 500 ppm (40.8 mg/kg/day) include delayed eye opening, delayed preputial separation in males, decreased motor activity counts for PND 14 females, and decreased caudate putamen measurements in the brains of adult female offspring. The NOAEL for these mid- and high-dose offspring effects is 20 ppm (1.7 mg/kg/day) with the exception of the decreased cauate putamen measurements, for which a NOAEL can not be established (due to lack of morphometry data for 20 ppm offspring).

This study is classified **Acceptable/non guideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6). This study may be upgradable if requested information is provided, including: diet concentration analyses, missing morphometric histopathology data (including bilateral measures used to generate mean values and all mid-dose data, and results of the original control measurements), procedural information for functional observation assessments, additional data for learning and memory evaluations, and adequate positive control data.

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:**   Flufenacet, technical grade
   Description: White powder
   Batch #:   603-0013
   Purity: 96.9% a.i. (April, 1998) and 96.0% (July, 1999)
   Compound Stability: Proven stable by reanalysis
   CAS # of TGAI: 142459-58-3
   Structure:

5
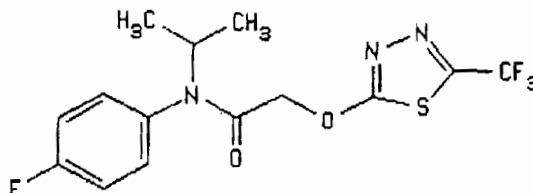
2. **Vehicle control:** The test article was administered in the feed, Purina Mills Rodent Lab Chow® #5001-4 in "etts" form, prepared using corn oil (Lot No. 15-12971) as the vehicle at 1% by weight of the diet and a small amount of acetone (Lot No. 14-07891) as a solvent.

3. **Test animals (P):**

Species:	Rat
Strain:	Crl:CD®BR VAF/Plus® (Sprague-Dawley)
Age at study initiation:	65 days old
Wt. at study initiation:	220-253 g
Source:	Charles River Laboratories, Inc., Raleigh, North Carolina
Housing:	Individually in stainless steel wire-bottomed cages until GD 20 then transferred to nesting boxes with Bed-o'cobs bedding. Pups were housed individually after weaning.
Diet:	PMI Diet #5001-4 "etts", *ad libitum*
Water:	Reverse osmosis processed tap water, *ad libitum*
Environmental conditions:	**Temperature:** 64-79°F (nominal)
	Humidity: 30-70% (nominal)
	Air changes: ≥10/hour, filtered
	Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	At least 8 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: July 22, 1998; End: October 30, 1998

2. **Study schedule:** The females were mated and assigned to study groups of 25 each. The test substance was administered in the feed to the maternal animals from gestation day (GD) 6 through lactation day 11. The day of completion of delivery for each litter was designated postnatal day (PND) 1 (or lactation day 1). Pups were weaned on postnatal day 22, after which time the dams were killed. F_1 animals remained on study up to approximately postnatal day 77.

3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily for the presence of sperm in a vaginal smear or a copulatory plug *in situ*. The day that evidence of mating was found was designated GD 0.

4. **Animal assignment:** Mated females were allocated to groups using a computer-generated randomization procedure based on body weights (Table 1). Randomly-selected pups were assigned to either neuropathological evaluation and brain weights on days 12 and 72-77, motor activity on days 14, 18, 22, and 60 and auditory startle habituation on days 23 and 61, passive avoidance on days 23-25 and 30-32 and water maze testing on days 59-62 and 66-69, or organ weight and blood sampling on days 12 and 22. Clinical observations were conducted on all pups daily during the preweaning period, and all live pups were evaluated daily for surface righting reflex from PND 1, pinna unfolding from PND 2, eye opening from PND 12, acoustic startle response from PND 13, and pupil constriction on PND 21 until the criterion was attained for all pups in the litter. Detailed clinical observations were conducted postweaning on Subset 4 offspring.

Table 1. Study design [a]

Experimental Parameter	Subgroup	Dose (ppm)			
		0	20	100	500
Maternal Animals					
No. of maternal animals assigned	NA	25	25	25	25
Detailed clinical observations (daily from GD 0 through LD 22)	NA	25	25	25	25
Offspring					
Clinical observations 　Preweaning (daily) 　Postweaning (weekly, detailed)	1-4 4	All 3/sex/litter	All 3/sex/litter	All 3/sex/litter	All 3/sex/litter
Developmental landmarks 　Surface righting, pinna unfolding 　Eye opening, acoustic startle, pupil constriction (PND 21) 　Sexual maturation	1-5 2-5 2-4	4/sex/litter 4/sex/litter 3/sex/litter	4/sex/litter 4/sex/litter 3/sex/litter	4/sex/litter 4/sex/litter 3/sex/litter	4/sex/litter 4/sex/litter 3/sex/litter
Motor activity (PND 14, 18, 22, 60)	3	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Auditory startle habituation (PND 23 and 61)	3	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Passive avoidance (PND 23-25 and retest 30-32)	2	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Watermaze (PND 59-63 and retest 66-69)	2	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Brain weight 　PND 12 　PND 68-79	1 4	1/sex/litter 6/sex	1/sex/litter 6/sex	1/sex/litter 6/sex	1/sex/litter 6/sex
Neuropathology 　PND 12 　PND 72-77	1 4	6/sex 6/sex	0 0	6/sex [b] 6/sex [b]	6/sex 6/sex
T3 & T4 　PND 12 　PND 22	1 5	1/sex/litter 1 ♂ or ♀ /litter	1/sex/litter 1 ♂ or ♀ /litter	1/sex/litter 1 ♂ or ♀ /litter	1/sex/litter 1 ♂ or ♀ /litter
Liver & thyroid weights 　PND 12 　PND 22	1 5	1/sex/litter 1/sex/litter	1/sex/litter 1/sex/litter	1/sex/litter 1/sex/litter	1/sex/litter 1/sex/litter

a　Obtained from page 26, MRID 45232501.
b　Examined due to treatment-related effects found at 500 ppm.

5. **Dose selection rationale:** Dose levels were chosen based on the results from a two-generation reproduction study (MRID 43850033) and a prenatal developmental toxicity study (MRID 43850030). In the reproduction study, male and female rats were administered the test article in the diet at concentrations of 0, 20, 100, or 500 ppm (0, 1.3, 7.0, and 36.1 mg/kg/day) through gestation. The high dose resulted in decreased maternal weight gain. The Agency review of this study describes increased liver weight in F1 females, hepatocytomegaly in F1 males, and pup death and cannibalism in both generations at the mid- and high-doses.

In the prenatal developmental toxicity study, female rats were administered 0, 5, 25, or 125 mg/kg/day by gavage on GD 6-15. Decreased maternal weight gain, decreased fetal body weight, delayed ossification of the fetuses, and increased incidences of extra ribs in the fetuses were found in the high-dose group. The registrant concluded that 125 mg/kg/day would be an excessive dose for developmental neurotoxicity testing.

On the basis of these studies, doses for the developmental neurotoxicity study were selected at 0, 20, 100, and 500 ppm.

6. **Dosage administration:** The test article was administered in the diet to dams on GD 6 through lactation day 11.

7. **Diet preparation and analysis:** Dietary mixtures were prepared by the sponsor and were used as received by the testing facility. Corn oil was used as the vehicle for the test substance at 1% by weight of the diet; a small amount of acetone served as a solvent in the diet preparation process and was allowed to evaporate. The mixtures were stored frozen and aliquots for weekly use were stored at room temperature. Homogeneity, stability, and concentration analyses of the dietary mixtures were conducted by the sponsor as part of the two-generation reproduction study; the results were included in the study report.

Results:
Homogeneity analysis: Mean concentrations of multiple samples from the top, middle, and bottom of 10 and 1000 ppm mixtures were within 15% of nominal with the coefficient of variation ≤ 8%.

Stability analysis: Test article concentrations in 10 and 1000 ppm mixtures were ±10% of their initial measured concentration after storage at room temperature for up to 14 days and ±15% after 51 days of freezer storage. The exceptions were samples analyzed after 28 days of freezer storage which were 124-125% of initial.

Concentration analysis: Absence of test article was confirmed in the feed. The overall mean concentrations of six dietary preparations of 20, 100, or 500 ppm were 94.5%, 103%, and 104%, respectively, of nominal.

The analytical data indicated that the test article could be mixed homogeneously in the feed and was stable for the duration of use. However, it is unlikely that the dietary mixtures

analyzed for concentration in the reproduction study were the same as those used in the current study (since the reproduction study was dated 1995).

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** All animals were checked twice daily for mortality and once for clinical signs of toxicity. Maternal behavior was evaluated daily when the pups were examined. Maternal behavior was recorded on lactation days 1, 5, 8, 14, and 22.

Beginning on GD 6, the dams were observed at approximately the same time each day by an individual who was unaware of the treatment group. The functional observations described below were recorded; however, the study report did not describe the procedures used for these observations, e.g., whether the same technicians were used throughout testing, where the testing was done (no mention was made as to whether animals were observed outside the home cage), when the testing was done with respect to time of dosing, the environmental conditions, whether a scoring or ranking system was used, or the duration of the observation period.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Assessment of lacrimation and salivation, and respiration 2) Presence or absence of piloerection, 3) Observations of urination and/or defecation, 4) Degree of palpebral closure and "prominence of the eye"
X	Incidence of abnormal movements.
X	Incidence of abnormal postures.
X	Incidence of abnormal behavior patterns and/or unusual appearance.

Individual maternal body weight and food consumption were measured on GD 0 and daily during the exposure and post-exposure intervals.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day, PND) 1. Litter size, live litter size, and pup viability at birth was determined. For pups that appeared stillborn or that died before the initial viability examination could be made, the lungs were removed and immersed in water to determine whether the pup was live born. The pups in each litter were counted daily. Clinical

9

observations were recorded daily during lactation and weekly thereafter. Body weights were assessed on lactation days 1, 5, 8, 12, 14, 18, and 22.

On day 5 postpartum, litters were standardized to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible); excess pups were killed and discarded. On PND 12, twenty litters/exposure group were randomly selected for continued examination.

2) **Developmental landmarks**: The following developmental landmarks were evaluated in all pups on the study. The number of pups that met the criterion was recorded on each day of testing. Testing continued until the day the criterion was attained by all pups in the litter.

> Surface righting reflex (ability to right in 5 seconds): from PND 1;
> Pinna unfolding: from PND 2;
> Eye opening: from PND 12;
> Acoustic startle response: from PND 13;
> Pupil constriction: once on PND 21.

Beginning on postnatal day 39, male offspring were examined daily for the age of balanopreputial separation. Beginning on postnatal day 28, female offspring were examined daily for the age of vaginal opening.

3) **Postweaning observations**: After weaning on postnatal day 22, offspring were examined daily for clinical signs of toxicity. Individual body weights and food consumption were recorded weekly.

4) **Neurobehavioral evaluations**: Observations and the schedule for those observations are summarized as follows from the report. One male and one female per litter (at least 20/sex/group) were randomly selected for each of the following tests. The same animals were used for passive avoidance and water maze testing and the same animals were used for motor activity and auditory startle habituation.

i) **Functional observations**: Standard clinical observations were conducted for preweaning offspring; no functional behavioral evaluations were conducted. For postweaning offspring, detailed clinical observations were conducted as described in the study report (p. 47): "An individual unaware of the maternal dosage group examined the Subset 4 rats weekly during the postweaning period for signs of autonomic dysfunction, abnormal postures, movements or behavior patterns, and unusual appearance, to the extent that these evaluations could be made at each age. Rats assigned to Subsets 2 and 3 were examined for gross signs of toxicity when they were weighed or removed from their cages for behavioral testing." Methods used for these evaluations were not described in any detail.

ii) **Motor activity testing**: Motor activity was evaluated in 1 pup/sex/litter/exposure group (subset 3) on PNDs 14, 18, 22, and 58-60; the same pups were evaluated each time. A passive infrared sensor mounted outside a stainless-steel 40.6 x 25.4 x 17.8 cm cage (with

10

Plexiglas® flooring during preweaning) was used to record the number of movements and time spent in movement over the course of a 1.5-hour session, with tabulation at each 5-minute interval. A rack of up to 32 cages and sensors was monitored during each session. Each rat was tested in the same location on the rack across test sessions, and groups were counterbalanced according to sex and treatment level across testing sessions and cages, where possible. No information was provided as to whether testing was performed at the same time of day across sessions.

iii) **Auditory startle habituation:** Auditory startle reflex habituation testing was performed on 1 pup/sex/litter/exposure group (subset 3) on PNDs 23 and 59-61, using a microcomputer to control the test session. Testing was conducted in a sound-attenuated chamber, using sets of 4 rats per session. Each rat was placed in a small cage above a platform that contained a force transducer in its base. There was an initial adaptation period of 5 minutes, and during the last minute of this period 10 "blank" trials were given to sample the baseline force in the absence of a stimulus. The rats were then given 50 trials of 30 msec, 120 dB bursts of noise at 10-second intervals, followed by an additional 10 "blank" trials. The microcomputer sampled the output of the force transducer and recorded the peak amplitude of each response. The response magnitude was calculated by subtracting the average response on baseline trials, and the average response magnitude and the pattern of responses over 10-trial blocks were compared among treatment groups.

iv) **Learning and memory testing:**

Passive avoidance: A passive avoidance test was conducted on PNDs 23-25 and again seven days later (PNDs 30-32); each animal was tested twice, with a one-week interval between test sessions. For each trial, the animal was placed in the "bright" compartment of a two-compartment chamber, the sliding door between compartments was opened, and the light was turned on. When the animal entered the "dark" compartment, the sliding door was closed, the light was turned off, and a 1 second pulse of 1 mA electric current was delivered to the grid floor of the compartment. The animal was then removed from the apparatus and placed in a holding cage for 30 seconds before the start of the next trial. The criterion for learning was that the rat remain in the "bright" compartment for 60 seconds on two consecutive trials, and trials were repeated until the criterion had been met or until 15 trials had been completed. For each trial the latency to enter the dark compartment was recorded.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the latency to enter the "dark" compartment on trial 1 of the first test session (activity levels and exploratory tendencies in a new environment); the latency to enter the "dark" compartment on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); and the latency to enter the "dark" compartment on trial 1 of the second session (long-term retention).

Water maze: Water maze testing was conducted on PND 59-62 and again seven days later (PNDs 66-69). Testing was conducted using a watertight, 16-gauge stainless-steel modified M-maze filled with $21\pm1°C$ water at a depth of approximately nine inches, and each animal was tested twice, with a one-week interval between test sessions. For each trial, the rat was placed in the starting position at the base of the M-maze, farthest from the two arms and required to swim to one of the two goals to be removed from the water. On the initial trial, the rat had to enter both arms of the maze before being removed from the water, and the first arm chosen was designated as the incorrect goal during the remaining trials of both test sessions. For each trial, the animals were given 60 seconds to make a correct goal choice, and animals failing to make a correct choice within that time were guided to the correct goal and then removed from the water. The inter-trial interval was 15 seconds. The criterion for learning was five consecutive errorless trials, and trials were repeated with a 15-second inter-trial interval until the criterion had been met or until 15 trials had been completed. For each trial, the latency to choose the correct goal and the number of errors, i.e., incorrect turns in the maze, were recorded. No information was provided regarding criteria for scoring errors.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the average number of errors for each trial on the first day of testing (for overall learning performance); the latency to reach the correct goal on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); the average number of errors for each trial in the second session (long-term retention); and the latency to reach the correct goal on trial 1 of the second session (long-term retention).

5) **Clinical chemistry**: Blood samples for analysis of serum T_3 and T_4 levels were taken at sacrifice from the vena cava of 20 pups/sex/group on PND 12 (subset 1) and from 10 pups/sex/group on PND 22 (subset 5).

2. Postmortem observations:

a. **Maternal animals**: Animals found dead or sacrificed during the study were subjected to gross necropsy of the thoracic, abdominal, and pelvic cavities. Maternal animals were sacrificed by carbon dioxide asphyxiation on postnatal day 22 if they delivered or presumed GD 25 if they failed to deliver a litter. Dams were subjected to gross necropsy and the number of implantation scars was recorded. The uterus of any animal judged to be non pregnant was stained with 10% ammonium sulfide solution. Dams with litters containing less than 9 pups were sacrificed on lactation day 12, subjected to gross necropsy, and the number of implantation sites were recorded. Any gross lesions were preserved in 10% formalin.

b. **Offspring**: Pups found dead at birth were evaluated for viability status as described previously. Pups found dead or sacrificed moribund during lactation and pups culled on PND 5 were subjected to gross necropsy. Gross lesions were preserved in Bouin's solution or 10% formalin. All pups were sacrificed as scheduled by carbon dioxide asphyxiation, except rats assigned for neuropathological examination on day 72-77. In

addition for rats sacrificed on PNDs 12 and 22, the liver and thyroid/parathyroid were retained in neutral buffered 10% formalin for histopathological examination and blood samples were collected from the vena cava for serum T_3 and T_4 concentrations. The liver was weighed at the time of collection and the thyroid/parathyroid was weighed at least 48 hours post fixation. Tissues were shipped to Research Pathology Services, Inc., New Britain, PA for histopathological processing and evaluation.

The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 12 (subset 1) or 72-77 (subset 4). These animals were subjected to postmortem examinations as described below.

On postnatal day 12, 20 pups/sex/group were selected for brain measurements. Animals were sacrificed by carbon dioxide asphyxiation followed by exsanguination from the inferior vena cava. The calvarium was removed to expose the brain and the entire head was immersed in neutral buffered 10% formalin. After approximately 48 hours, the brains were weighed and returned to fixative. The brains of 6 pups/sex/group were shipped to Experimental Pathology Laboratories, Inc. (EPL), Herndon, VA for histological processing; histopathological evaluation was conducted by Consultants in Veterinary Pathology, Murrysville, PA.

Prior to sectioning, a Vernier caliper was used to measure 1) the length of the cerebrum from the anterior to posterior pole, exclusive of the olfactory bulbs; and 2) a linear measurement of the cerebellum extending from the anterior edge of the cortex to the posterior pole. The brains were then cut into six coronal slices, by means of the following cuts: 1) half-way between the ventral base of the olfactory bulbs and the optic chiasm; 2) through the optic chiasm; 3) through the infundibulum; 4) through the midbrain just posterior to the mammillary body; 5) through the cerebellum just anterior to its mid point, and 6) through the anterior portion of the medulla. The brain slices were processed according to standard operating procedures for paraffin embedding. The tissue blocks were sectioned on a rotary microtome at a thickness of 6 μ and stained with hematoxylin and eosin. Qualitative histopathological examination was performed on tissues from control and high-dose animals. In addition, the following linear opic measurements were taken, using a calibrated ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex (the dorsolateral portion of the cerebral cortex) within the coronal section taken through the optic chiasm; 3) diagonal width (maximum cross-sectional width) of the caudate putamen and underlying globus pallidus; 4) thickness of the corpus callosum at its mid point within the section taken at the level of the optic chiasm; 5) thickness of the dorsal to lateral portion of the dentate gyrus of the hippocampus within the section taken through the hypothalamus; 6) the height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface, and 7) thickness of the external germinal layer of the cerebellum (multiple areas were measured and the median value recorded as one measurement). For those areas measured bilaterally, only the mean was provided in the data report.

13

Initially, morphometric evaluations were conducted only for control and high-dose groups. After the initial analysis of the morphometry data, a decision was made to analyze the brains of the intermediate dose pups. The intermediate dose brains were sectioned and evaluated without knowledge of treatment group. Due to "inconsistent section orientation between these additional brains and those originally processed from the control and high-dose groups, all brains were re-measured at the same time. Only the data from the second evaluation were presented in the study report although apparently all data are retained in the histopathology raw data for the study.

At postnatal day 72-77, the 6 animals/sex/group selected for neurohistopathological evaluation were administered a combination of heparin and sodium pentobarbital and perfused *in situ* with neutral buffered 10% formalin. All animals were subjected to necropsy and gross lesions were preserved in neutral buffered 10% formalin.

The calvarium was removed to expose the brain and the entire head was placed in the fixative. After approximately 48 hours, the brains were weighed and returned to fixative. The vertebral column and hind limbs were dissected to expose the spinal cord and peripheral nerves, and were then immersed in neutral buffered 10% formalin. Tissues from all control and high-dose animals were shipped to Experimental Pathology Laboratories, Inc. (EPL), Herndon, VA for histological processing; histopathological evaluation was conducted by Consultants in Veterinary Pathology, Murrysville, PA.

Prior to sectioning, a Vernier caliper was used to measure 1) the length of the cerebrum from the anterior to posterior pole, exclusive of the olfactory bulbs; and 2) a linear measurement of the cerebellum extending from the anterior edge of the cortex to the posterior pole. The brains were then cut into nine coronal slices, by means of the following cuts: 1) just posterior to the olfactory bulbs, 2) midway between the optic chiasm and the plane of the first slice, 3) through the optic chiasm; 4) through the infundibulum; 5) at the posterior edge of the mammillary body; 6) just in front of the anterior edge of the pons, 7) just anterior to the middle of the cerebellar cortex, 8) through the posterior portion of the cerebellar cortex, and 9) through the anterior portion of the medulla. The brain slices were processed according to standard operating procedures for paraffin embedding. The tissue blocks were sectioned on a rotary microtome at a thickness of 5 μ and stained with hematoxylin and eosin. Qualitative histopathological examination was performed on tissues from control and high-dose animals. In addition, the following linear optic measurements were taken, using a calibrated ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex (the dorsolateral portion of the cerebral cortex) within the coronal section taken through the optic chiasm; 3) diagonal width (maximum cross-sectional width) of the caudate putamen and underlying globus pallidus; 4) thickness of the corpus callosum at its mid point within the section taken at the level of hippocampal fimbria or at the level of the dorsal anterior portion of the hippocampal gyrus; 5) thickness of the dorsal to lateral portion of the dentate gyrus of the hippocampus within the section taken at the level of the hypothalamus (infundibulum) and mammillary body; and 6) the height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth

14

ventricle to the dorsal surface of the cerebellar cortex. For those areas measured bilaterally, only the mean was provided in the data report.

Initially, morphometric evaluations were conducted only for control and high-dose groups. After the initial analysis of the morphometry data, a decision was made to analyze the brains of the intermediate dose animals, which had been held in fixative for 8 additional months. The intermediate dose brains were sectioned and evaluated without knowledge of treatment group. At the same time, the previously measured brain sections were re-measured in blinded fashion to "assure that the most homologous sections were compared." Finally, a third set of striatal measurements were taken from all of the adult female brains, including a repeat of the diagonal measurements and the transverse measurements. Digital scans were taken on the striatal-level sections, and animals with non-homologous sections were excluded. The study report included all control and high-dose measurements, but only included the brain weights, gross measurements, and striatal measurements for the intermediate dose females. The male data are not presented in the study report; because of "a combination of inconsistent section level" for this group and of "prolonged fixation in formalin for these brains," the data for the intermediate dose groups were considered to be unreliable. The data are reported to be retained in the histopathology raw data for the study.

The following central and peripheral nervous tissues (X) were dissected, embedded in paraffin (CNS tissues) or glycol methacrylate (PNS tissues), blocked, sectioned, and stained with hematoxylin and eosin, Bielschowsky's technique, and luxol fast blue/cresyl violet (paraffin tissue blocks, 5 micrometer sections) or hematoxylin and eosin, Bielschowsky's technique, and toluidine blue (glycol methacrylate blocks, 2 micrometer sections). Neurohistological evaluation was performed on tissues from males and females in the control and high dose groups.

The CHECKED (X) tissues were evaluated

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Olfactory bulbs	X	Sciatic (cross- and longitudinal sections) *
X	Optic nerve/chiasm *	X	Tibial (cross- and longitudinal sections) *
X	Cerebral cortex	X	Common peroneal (longitudinal section) *
X	Hippocampus	X	Sural (longitudinal section) *
X	Basal ganglia		
X	Thalamus		
X	Hypothalamus		
X	Midbrain		
X	Cerebellum		
X	Pons		
X	Medulla oblongata		

15

	SPINAL CORD		OTHER
X	Cervical (cross and longitudinal sections) *	X	Dorsal root ganglia (longitudinal sections) *
X	Thoracic (cross and longitudinal sections) *	X	Spinal nerve roots (longitudinal sections) *
X	Lumbar *		
	OTHER		
X	Gasserian ganglion *		
X	Trigeminal nerves *		

Data taken from Appendices K and L, pp. 761 ff and 800 ff, MRID 45232501.
* Examined in adult offspring only.

D. DATA ANALYSIS

1. **Statistical analyses:** Body weights, food consumption, latency and errors per trial scores in behavioral tests and percent mortality per litter were analyzed with Bartlett's Test for homogeneity of variance. If Bartlett's test was significant, the data were analyzed by nonparametric means; if Bartlett's test was not significant the data were analyzed by ANOVA followed by Dunnett's test. For nonparametric data, when $\leq 75\%$ of scores in all groups were tied, the Kruskal-Wallis test was used followed by Dunn's test; if $>75\%$ of the scores in all groups were tied, Fisher's Exact test was used to compare the proportion of ties in the dosage groups. Clinical observation data, as well as other proportion data, were analyzed as contingency tables using the Variance Test for Homogeneity of the Binomial Distribution.

   Data from motor activity and auditory startle habituation tests, with measurements recorded at intervals (blocks), were analyzed using an ANOVA with Repeated Measures. If the dosage effect was significant, the totals for the control group and the groups given the test substance were compared using Dunnett's test. If the dosage x block interaction was significant, an ANOVA was used to evaluate the data at each measurement period followed by Dunnett's test.

2. **Indices:**

   a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

      Pregnancy rate = (No. rats pregnant/No. rats mated) × 100

      Gestation index = (No. live litters born/No. pregnant rats) × 100

   b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

14

Viability index = (No. of live pups on PND 5 precull/No. live pups on PND 1) × 100

Lactation index = (No. live pups on Day 22/No. live pups on PND 5 post cull) × 100

3. Historical control data: Summary historical (negative) control data were provided in a supplemental submission (MRID 45796117). These included the following endpoints: functional observational battery testing in adult rats, preweaning developmental landmarks, sexual maturation, motor activity (PND 14, 18, 21, and 60), auditory startle habituation (for PND 22-23 and 60), passive avoidance (primarily in adults but with minimal data from PND 24 or 42 weanlings), water maze (apparently only in adults), brain weights (adults), and morphometric measurements (PND 12 and adult).

4. Positive control data: Positive control data for neurobehavior and neuropathology were presented in the study report, and are summarized in Attachment 1 to this DER. Most of the positive control studies are unacceptable for use with the current study. Few of the studies were conducted within the last few years before the current study. The majority of the studies did not utilize immature rats as test subjects. None of the studies that included motor activity assessment used a 1.5-hour session with 5-minute blocks. Few of the studies included complete descriptions of the methods used or tables of individual data. None of the studies demonstrated the laboratory's ability to detect major functional neurotoxic endpoints using the observational methods used in the current study.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** All animals survived to scheduled termination. No treatment-related clinical signs of toxicity were observed during gestation or lactation. Common findings at low incidences (1 to 3 rats per group) in control and treated groups (with no dose-response relationship) included excess salivation, localized alopecia, red or brown perivaginal substance, and urine-stained abdominal fur.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 2. Values for the low-dose group were similar to the controls during gestation and lactation. During gestation, transient decreases in maternal body weight, body weight gain, and food consumption were observed in the mid- and high-dose groups (100 and 500 ppm) following the initiation of dietary treatment. Mean absolute body weights for the mid- and high-dose groups were significantly ($p \le 0.05$ or 0.01) less than those of the control group on days 8-9 (96% of control) and days 8-12 (93-96% of control), respectively. Significantly reduced ($p \le 0.05$ or 0.01) mean body weight gains were observed for the mid-dose group during GD 6-9 (47% of control) and for the high-dose group for the intervals of GD 6-9 (37% of control) and 6-21 (86% of control). Decreases in mean body weight gains correlated with significantly ($p \le 0.01$) reduced mean food consumption during GD 6-9 for the mid-dose group (84% of control) and GD 6-12 for the high-dose group (72-84% of control) as compared with the

17

controls. Mean relative food consumption (g/kg/day) was also decreased significantly
($p \leq 0.01$) for the mid-dose group on GD 6-9 and for the high-dose group on GD 6-9 and 9-12.
Both the mid- and high-dose groups showed slightly or significantly greater absolute and
relative food consumption during GD 12-15, resulting in slightly greater mean body weight
gains by these groups for this interval; this suggests an adaptation to the treated feed.

During lactation, the only significant differences in mean absolute body weights were lower
mean weights for the high-dose group on days 8 and 9 ($p \leq 0.05$; 95-96% of controls). Mean
body weight gains for the high-dose group were greater than those of the controls throughout
most of lactation with significance ($p \leq 0.01$) attained for the interval of days 7-12. Mean
food consumption for the treated groups was similar to the controls throughout lactation with
one exception - during days 7-12 food consumption for all treated groups was significantly
($p \leq 0.05$ or 0.01) less than that of the control, but the effect was not dose-related.

Observations/study interval	Dietary concentration (ppm)			
	0	20	100	500
TABLE 2. Selected mean (±SD) maternal body weight and food consumption data [a]				
Gestation (n = 22-25)				
Body wt. Gestation day 0 (g)	235.4 ± 8.2	235.2 ± 8.1	235.8 ± 8.2	236.5 ± 8.2
Body wt. Gestation day 6 (g)	265.9 ± 12.6	267.6 ± 12.4	264.9 ± 8.9	267.3 ± 11.1
Body wt. Gestation day 9 (g)	284.9 ± 16.1	281.4 ± 18.1	273.9* ± 15.2 (96)	274.3** ± 14.6 (96)
Body wt. Gestation day 15 (g)	318.2 ± 20.1	314.8 ± 18.1	310.2 ± 14.0	311.2 ± 16.8
Body wt. Gestation day 20 (g)	382.0 ± 22.5	378.4 ± 25.7	375.4 ± 22.0	375.0 ± 26.1
Wt. gain Gestation days 0-6 (g)	30.5 ± 7.4	32.3 ± 7.8	29.1 ± 5.7	30.8 ± 8.9
Wt. gain Gestation days 6-9 (g)	19.0 ± 6.1	13.8 ± 12.2	9.0** ± 13.6 (47)	7.0** ± 10.0 (37)
Wt. gain Gestation days 6-21 (g)	135.3 ± 16.2	122.4 ± 23.2	124.4 ± 22.7	116.2** ± 18.0 (86)
Food consumption gestation days 0-6 (g/day)	22.3 ± 2.0	22.7 ± 2.0	22.2 ± 1.8	22.8 ± 2.1
Food consumption gestation days 6-9 (g/day)	25.7 ± 3.3	24.2 ± 3.9	21.5** ± 5.4 (84)	18.6** ± 4.2 (72)
Food consumption gestation days 6-9 (g/kg/day)	92.9 ± 9.3	87.6 ± 11.9	79.4** ± 18.3 (85)	69.6** ± 14.8 (75)
Food consumption gestation days 9-12 (g/kg/day)	89.9 ± 7.6	85.2 ± 8.6	89.9 ± 8.8	78.7** ± 11.8 (88)
Food consumption gestation days 12-15 (g/kg/day)	85.4 ± 6.9	86.0 ± 6.5	89.4 ± 10.3	98.0** ± 6.7 (115)
Food consumption gestation days 6-21 (g/day)	26.8 ± 2.4	26.0 ± 1.8	25.9 ± 2.5	25.1 ± 2.4
Food consumption gestation days 6-21 (g/kg/day)	84.8 ± 5.4	83.5 ± 2.9	82.7 ± 5.7	81.6 ± 5.5
Lactation (n = 22-25 thru LD 12, n = 20 thereafter)				
Body wt. lactation day 1 (g)	282.7 ± 18.5	283.3 ± 17.4	279.8 ± 16.1	275.5 ± 21.6
Body wt. lactation day 6 (g)	294.3 ± 17.6	295.4 ± 20.5	289.2 ± 19.3	283.7 ± 19.6
Body wt. lactation day 12 (g)	318.6 ± 24.7	323.4 ± 25.6	316.8 ± 14.7	316.6 ± 19.3
Body wt. lactation day 18 (g)	325.8 ± 15.0	329.6 ± 22.1	323.6 ± 14.1	323.6 ± 20.4
Body wt. lactation day 22 (g)	319.4 ± 14.7	331.8 ± 23.3	326.0 ± 14.7	326.6 ± 26.4
Wt. gain lactation days 1-12 (g)	35.9 ± 15.1	39.8 ± 23.4	37.1 ± 12.5	41.1 ± 17.2
Wt. gain lactation days 12-22 (g)	0.4 ± 18.7	11.8 ± 20.0	11.3 ± 16.6	11.1 ± 21.0
Food consumption lactation days 1-12 (g/day)	48.1 ± 5.6	45.0 ± 6.9	45.1 ± 6.4	44.2 ± 4.3
Food consumption lactation days 12-22 (g/day)	65.3 ± 11.5	64.5 ± 6.3	64.0 ± 7.7	63.6 ± 10.0

[a] Data obtained from Tables B3-B7 and B9, pp. 128-134 and 136, MRID 45232501.
Percent of mean control value is presented in parentheses; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

3. **Test substance intake:** Based on maternal food consumption and body weight data and nominal dietary concentrations. Average doses during gestation and lactation are given in Table 3.

19

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day) [a]			
Period	Dietary concentration (ppm)		
	20	100	500
Gestation days 6-21	1.7 ± 0.1	8.3 ± 0.6	40.8 ± 2.8
Lactation days 1-12	3.0 ± 0.4	15.4 ± 1.9	76.7 ± 6.7

[a]Data obtained from Table B1, pp. 124-125, MRID 45232501.

4. **Reproductive performance:** Results for the maternal animals are summarized in Table 4. The gestation index, length of gestation, and number of implantation sites were similar between the treated and control groups. The pregnancy rate was significantly ($p \le 0.01$) reduced in the high-dose group compared with the control group. The study author did not consider this effect to be treatment-related because pregnancy was determined prior to initiation of treatment, and because the incidence was within the historical control range for the testing facility. The possibility of late preimplantation loss could not be evaluated since corpora lutea data were not counted. The number of dams with stillborn pups was significantly ($p \le 0.01$) less than the controls for all treated groups due to a large number of affected dams in the control group. One control dam failed to deliver and was sacrificed on GD 25.

TABLE 4. Reproductive performance [a]				
Observation	Dietary concentration (ppm)			
	0	20	100	500
Number Mated	25	25	25	25
Number (%) Pregnant	25 (100)	25 (100)	25 (100)	22 (88) **
Pregnancy Rate (%)	100	100	100	88
Number Delivered	24	25	25	22
Gestation Index (%)	96	100	100	100
Gestation Length (days)	22.7 ± 0.5	22.6 ± 0.6	22.8 ± 0.5	22.9 ± 0.4
Mean Number of Implantation Sites	16.3 ± 1.4	15.0 ± 3.2	15.0 ± 3.0	16.4 ± 1.9
Number (%) with Stillborn Pups	7 (29.2)	3 (12.0)**	2 (8.0)**	0 (0)**
Number with Complete Litter Loss	0	0	0	0

[a]Data obtained from Table B11, p. 138, MRID 45232501.
Significantly different from control: **$p \le 0.01$.

5. **Maternal postmortem results:** No treatment-related gross lesions were found in any female at necropsy. The control dam which failed to deliver was found to have 5 live and 5 dead fetuses *in utero* and one partially delivered live fetus.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) and pup reflex and physical development during lactation are summarized in Table 5. No treatment-related effect was observed on the number of litters, live litter size, sex ratios at birth, or pup survival. The number of pups found dead or presumed cannibalized was increased ($p \le 0.01$) for the mid-dose group during days 6-8 mainly due to one litter with seven dead or missing pups. A corresponding decrease in the number of live pups/litter was found in the mid-dose group on day 8, but no dose-related pattern was observed. No treatment-related clinical signs of toxicity were observed in any pup during lactation. Incidental findings included: thin, cold to touch, not nursing, lesion on head, small, or tip of tail black or missing.

During the first week after weaning (Week 4 postpartum), 1, 2, 3, and 5 deaths were noted for the 0, 20, 100 and 500 ppm groups, respectively. One additional 20 ppm F1 female died in Week 7 postpartum. Although the incidence of postweaning deaths is suggestive of a treatment-related effect, the study author considered these deaths to be incidental to treatment. No treatment-related clinical signs of toxicity were observed in any animal during the post weaning interval.

TABLE 5. Litter size and viability [a]				
Observation	Dietary concentration (ppm)			
	0	20	100	500
Number of Litters	24	25	25	22
Sex ratio at birth (% male)	49.5	45.8	50.3	47.9
Mean number stillborn	0.4 ± 0.7	0.1 ± 0.3	0.1 ± 0.3	$0.0* \pm 0.0$
Mean number live pups				
Day 1	15.1 ± 1.8	13.8 ± 3.2	13.8 ± 2.8	15.0 ± 2.1
Day 5 [b]	14.8 ± 1.9	13.5 ± 3.4	13.3 ± 2.7	14.5 ± 2.2
Day 5 [c]	10.0 ± 0.0	9.6 ± 1.5	9.8 ± 0.8	10.0 ± 0.0
Day 22	7.5 ± 1.6	7.9 ± 0.3	7.7 ± 0.7	7.7 ± 0.9
No. dead/missing pups (litters)				
Stillborn	10 (7)	3 (3)	2 (2)	0 (0)
Day 1	2 (2)	1 (1)	3 (3)	2 (1)
Days 2-5	7 (6)	10 (6)	13 (7)	9 (7)
Days 6-12	2 (2)	3 (3)	9 (3)	1 (1)
Days 13-21	8 (3)	0 (0)	4 (2)	2 (2)
Postweaning	1	3	3	5
Viability index day 5 (%)	97.5	96.7	95.5	96.7
Lactation index day 22 (%) [c]	62.5	65.8	67.2	70

[a] Data obtained from Table B12, pp. 139-142, Table B22, pp. 191-198, and Table C12-C13, pp. 308-333, MRID 45232501.
[b] Before standardization (culling).
[c] After standardization (culling).
[d] Calculations not adjusted for the removal of PND 11 pups (6/sex/dose) for neuropathology evaluation.
Significantly different from control: *$p \le 0.05$.

2. **Body weight:** Selected mean pup body weights and weight gains during lactation are summarized in Table 6. Mean absolute body weights were comparable at birth across all dose groups. Mean body weights of pups from the treated groups were significantly ($p \leq 0.05$ or 0.01) less than the controls beginning on day 5 for the high-dose males and females, day 12 for the mid-dose males and females, and day 12 or 14 for the low-dose males and females. Weight gains for the treated pups were significantly ($p \leq 0.05$ or 0.01) reduced during most intervals, with the most pronounced effects during lactation days 5-12.

TABLE 6. Mean (±SD) pre-weaning pup body weights and weight gains (g) [a]				
Postnatal Day	**Dietary concentration (ppm)**			
	0	**20**	**100**	**500**
Absolute body weights				
1	6.3 ± 0.4	6.2 ± 0.6	6.4 ± 0.6	6.1 ± 0.5
5 (precull)	8.8 ± 0.8	8.8 ± 1.0	9.0 ± 1.6	8.4 ± 1.0
5 (post cull) - males - females	9.3 ± 0.9 8.8 ± 0.9	9.2 ± 0.9 8.7 ± 1.0	9.4 ± 1.1 8.8 ± 1.1	8.6** ± 0.9 (92)[b] 8.0** ± 1.0 (91)
12 - males - females	20.4 ± 3.0 19.5 ± 2.8	19.0 ± 3.3 18.4* ± 2.8 (94)	18.5** ± 3.0 (91) 17.8** ± 3.1 (91)	17.4** ± 4.0 (85) 16.8** ± 3.9 (86)
18 - males - females	34.5 ± 4.8 33.3 ± 4.5	30.5** ± 3.2 (88) 29.7** ± 3.5 (89)	30.2** ± 4.0 (88) 29.4** ± 4.4 (88)	30.7** ± 7.3 (89) 29.5** ± 7.2 (89)
22 - males - females	47.2 ± 6.3 44.8 ± 6.4	42.0** ± 4.5 (89) 40.9** ± 5.2 (91)	41.4** ± 5.5 (88) 40.1** ± 6.2 (90)	41.3** ± 8.9 (88) 39.7** ± 8.8 (89)
Body weight gain				
5-12 - males - females	11.1 ± 2.5 10.7 ± 2.5	9.7* ± 3.1 (87) 9.6* ± 2.6 (90)	9.1** ± 2.6 (82) 9.0** ± 2.6 (84)	8.9** ± 3.4 (80) 8.7** ± 3.3 (81)
5-22 - males - females	37.9 ± 6.0 36.0 ± 6.1	32.8** ± 4.1 (87) 32.2** ± 4.8 (89)	32.1** ± 5.2 (85) 31.3** ± 5.7 (87)	32.7** ± 8.4 (86) 31.7** ± 8.1 (88)

[a] Data obtained from Table B12, p. 143, Table C3 - C6, pp. 298-303, MRID 45232501.
[b] Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

Selected mean post weaning offspring body weight data are presented in Table 7. Body weights for the treated groups were significantly ($p \leq 0.05$ or 0.01) less than the control group throughout the study for the mid- and high-dose males and high-dose females, until PND 51 for the mid-dose females, and until PND 30 for the low-dose males and females. Body weights for the mid-dose males and high-dose animals were 83-85% of the control levels after weaning and gradually increased to 95-96% of the controls by the end of the study. Body weights of the mid-dose females and low-dose animals also recovered to the control levels until significance was no longer observed. Body weight gains for the mid-dose males and the high-dose males and females were 90-94% (all $p \leq 0.01$) of the control levels during the first two weeks of the post weaning period. No other differences in body weight gains were observed between the treated and control groups during the post weaning interval.

Mean absolute food consumption was significantly ($p \leq 0.05$ or 0.01) reduced for all treated males and females during the first week of the post weaning interval compared with that of the controls. In addition, mean food consumption was significantly ($p \leq 0.05$ or 0.01) less

than the controls for the mid- and high-dose males during the second and third weeks and for the high-dose females during the third week of the post weaning interval. However, relative food consumption (grams feed consumed per kg body weight per day) was unaffected or increased, for treated groups as compared to control for this period (Tables C7-C10, pp. 305-308, MRID 45232501), indicating that observed postweaning body weight deficits were not attributable to reduced food consumption.

PND	TABLE 7. Mean (±SD) post-weaning pup body weights and weight gains (g) [a]			
	Dietary concentration (ppm)			
	0	20	100	500
Males				
23	49.4 ± 8.3	43.2** ± 5.5 (87)[b]	42.0** ± 6.9 (85)	41.0** ± 10.3 (83)
30	94.1 ± 13.6	87.2* ± 9.8 (93)	83.0** ± 12.2 (88)	81.8** ± 16.1 (87)
44	217.0 ± 23.3	208.4 ± 19.5	202.3** ± 22.2 (93)	199.0** ± 27.6 (92)
51	273.5 ± 28.1	263.8 ± 23.1	256.2** ± 25.1 (94)	254.0** ± 30.6 (93)
58	332.9 ± 33.1	323.2 ± 28.0	315.5** ± 30.4 (95)	310.8** ± 34.3 (93)
65	380.7 ± 36.1	371.9 ± 31.1	365.8* ± 33.0 (96)	358.9** ± 38.8 (94)
72	415.0 ± 39.8	408.4 ± 32.4	399.9* ± 35.6 (96)	394.1** ± 34.7 (95)
23-72	366.0 ± 34.8	365.0 ± 30.5	357.7 ± 32.9	352.4 ± 29.2
5-72	405.7 ± 39.4	399.2 ± 32.0	390.5* ± 35.1 (96)	385.5** ± 34.1 (95)
Females				
23	46.9 ± 7.4	42.1** ± 6.9 (90)	40.7** ± 7.5 (87)	40.0** ± 10.4 (85)
30	85.6 ± 11.0	80.4* ± 9.2 (94)	78.6** ± 10.0 (92)	75.1** ± 13.3 (88)
44	164.1 ± 14.4	161.4 ± 14.4	156.6* ± 13.6 (95)	151.2** ± 17.1 (92)
51	190.0 ± 15.4	187.0 ± 16.4	182.0* ± 13.9 (96)	176.9** ± 17.1 (93)
58	212.9 ± 17.7	211.8 ± 20.2	206.0 ± 15.1	200.2** ± 20.1 (94)
65	231.8 ± 19.0	232.2 ± 22.9	226.0 ± 16.7	220.4** ± 23.2 (95)
72	246.9 ± 19.5	247.9 ± 24.6	239.8 ± 17.4	235.0** ± 23.9 (95)
23-72	199.5 ± 17.4	205.5 ± 22.9	198.5 ± 17.3	193.9 ± 21.0
5-72	238.0 ± 19.2	239.2 ± 24.3	231.0 ± 17.3	226.8** ± 23.5 (95)

[a] Data obtained from Tables C3 - C6, pp. 298-304, MRID 45232501.
[b] Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

3. **Developmental landmark data:** Preweaning developmental landmark and postweaning sexual maturation data are presented in Tables 8A and 8B, respectively. The average day of eye opening was significantly (p≤0.05 or 0.01) delayed in pups from the mid- and high-dose groups compared with the controls. Mean days to attainment of other reflex and physical development endpoints, such as surface righting, pinna unfolding, acoustic startle, or pupil constriction, were similar between the treated and control groups.

23

The average day of preputial separation was delayed in mid- and high-dose males by approximately one day (p≤0.05) compared with the controls. No effect on the age of vaginal patency was observed in treated females.

TABLE 8A. Developmental landmarks (days postpartum) [a]				
Observation	Dietary concentration (ppm)			
	0	20	100	500
Surface righting[b]	4.8 ± 1.4	4.2 ± 2.2	4.0 ± 2.8	3.3 ± 2.1
Pinna unfolding[b]	3.8 ± 0.5	3.8 ± 0.7	3.8 ± 0.8	4.0 ± 0.8
Eye opening[b]	14.8 ± 0.7	15.3 ± 0.9	15.4* ± 0.8	15.6** ± 0.7
Acoustic startle[b]	13.3 ± 0.7	13.4 ± 0.7	13.4 ± 0.7	14.0 ± 1.2

[a]Data obtained from Table B14, pp. 145-148, MRID 45232501.
[b]The average day postpartum that at least 50% of the pups had the developmental measure present.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 8B. Mean (±SD) age of sexual maturation (days) [a]				
Endpoint	Dietary concentration (ppm)			
	0	20	100	500
Preputial separation (males)	47.2 ± 2.4	47.7 ± 1.9	48.4* ± 2.4	48.4* ± 2.7
Vaginal opening (females)	32.0 ± 2.0	31.5 ± 2.4	32.4 ± 2.3	32.5 ± 2.8

[a]Data obtained from Table C11, p. 309, MRID 45232501.
Significantly different from control: *p ≤ 0.05.

4. **Clinical chemistry:** Serum T_3 and T_4 levels for PND 12 and 22 offspring are summarized in Table 9. No effect of treatment was evident.

TABLE 9. Mean (±SD) serum T3 and T4 levels [a]					
Parameter		Dietary concentration (ppm)			
		0	20	100	500
Males					
T3 (μg/dL)	PND 12	0.64 ± 0.169 (n=9)	0.56 ± 0.206 (n=8)	0.58 ± 0.215 (n=8)	0.62 ± 0.313 (n=7)
	PND 22	1.13 ± 0.138 (n=10)	1.11 ± 0.200 (n=10)	1.13 ± 0.154 (n=10)	1.15 ± 0.129 (n=10)
T4 (ng/mL)	PND 12	2.27 ± 1.113 (n=20)	2.22 ± 0.902 (n=19)	2.05 ± 1.024 (n=20)	2.19 ± 0.841 (n=18)
	PND 22	3.66 ± 0.686 (n=10)	4.73 ± 0.607 (n=10)	3.98 ± 1.004 (n=10)	4.57 ± 1.183 (n=10)
Females					
T3 (μg/dL)	PND 12	0.67 ± 0.121 (n=10)	0.60 ± 0.095 (n=9)	0.58 ± 0.177 (n=10)	0.59 ± 0.136 (n=9)
	PND 22	1.48 ± 1.377 (n=10)	1.16 ± 0.141 (n=10)	1.24 ± 0.166 (n=10)	1.25 ± 0.132 (n=10)
T4 (ng/mL)	PND 12	2.37 ± 1.033 (n=18)	2.07 ± 0.842 (n=17)	1.88 ± 0.740 (n=18)	2.49 ± 0.865 (n=17)
	PND 22	3.97 ± 1.488 (n=10)	4.64 ± 1.090 (n=10)	3.93 ± 1.098 (n=10)	4.33 ± 0.813 (n=10)

[a] Data obtained from Appendix P, p. 926, MRID 45232501.

24

5. **Behavioral assessments:**

a. **Functional observations:** With the exception of delayed eye opening in the mid- and high-dose pups (see above), standard clinical observations during lactation, and detailed clinical observations of postweaning offspring did not reveal any treatment-related effects.

b. **Motor activity:** Total activity count data are summarized in Table 10A; these data were paralleled by the mean time spent in movement (not presented in DER). Coefficients of variation for the control group were 44-66% for PND 14, 39-55% for PND 18, 52-54% for PND 22, and 25% for PND 60. At each testing session there were some animals that moved very little, and some that were continuously moving. Habituation was not observed in subsession data on PND 14 and 18. Some habituation was evident in both male and female offspring on PND 22, and was more pronounced on PND 60.

For males, no significant differences were found between the treated and control groups in total activity or time spent in movement on any testing day. Activity levels in the treated males for an individual trial or block were occasionally significantly greater than or less than the control value, but no dose- or time-related pattern was apparent. On PND 14, high-dose females had significantly ($p \leq 0.01$) fewer total number of movements than the controls (a 51% decrease). A 34% reduction (n.s) in the total number of movements was also observed in PND 14 mid-dose females. At the low-dose, the number of movements was reduced only 20%.

An examination of the individual PND 14 subsession data (Table 10B) shows a predominance of very low (≤ 200) activity counts in the mid- and high-dose female pups. In addition, there appeared to be a generalized decrease in the individual PND 14 activity counts for male pups across all treated groups, but with no clear dose response. Notably, delayed development was observed in all treated groups during lactation (as demonstrated by significantly decreased body weight), and significant delays in eye opening were noted in the mid- and high-dose pups during the lactation period. These findings, particularly the delayed eye opening, are consistent with and support the interpretation of a treatment-related effect on motor activity for mid- and high-dose females at PND 14. Other differences between the treated and control females were sporadic, occurred for a single block, and were not dose-related.

Subsession data for PND 14 females demonstrated a significant reduction ($p \leq 0.05$ or 0.01) in movement during blocks 9-12 and 16-18 (Table 10C). In addition, the low- and mid-dose females also had reduced movement ($p \leq 0.05$ or 0.01) during blocks 10 (mid-dose only), 11, 12, and 18. Correspondingly the time spent in movement was significantly reduced for all treated females for blocks 11, 12, and 18.

In summary, evidence of a treatment-related effect on mid- and high-dose PND 14 female pup motor activity was demonstrated by a dose-dependant decrease in mean motor activity counts which achieved significance at the high-dose, a predominance of very low

activity counts in the individual pup activity count data, and corroborative evidence of significant decreases in mean motor activity during various testing subsessions.

TABLE 10A. Mean (±S.D.) motor activity data (total number of movements) [a]				
Test Day	Dietary concentration (ppm)			
	0	20	100	500
Males (n = 19-20)				
PND 14	465.0 ± 305.0 [66]	373.7 ± 304.5	293.2 ± 332.4	338.0 ± 393.1 (73)
PND 18	764.3 ± 421.9 [55]	925.4 ± 426.7	622.0 ± 362.0	652.7 ± 324.0 (85)
PND 22	636.4 ± 331.8 [52]	801.4 ± 295.4	747.2 ± 306.7	658.9 ± 363.6
PND 60	843.1 ± 213.0 [25]	837.8 ± 226.4	763.2 ± 267.6	747.3 ± 241.6 (89)
Females (n = 19-20)				
PND 14	610.7 ± 268.7 [44]	488.2 ± 303.8 (80)	401.1 ± 311.8 (66) [78]	299.1** ± 352.1 (49) [118]
PND 18	932.2 ± 365.5 [39]	942.2 ± 317.2	849.4 ± 376.3 (91) [44]	726.4 ± 401.6 (78) [55]
PND 22	684.8 ± 369.5 [54]	682.2 ± 282.6	823.2 ± 233.5	670.8 ± 373.6 [56]
PND 60	961.8 ± 239.5 [25]	965.3 ± 203.3	887.4 ± 220.4	938.0 ± 192.9

[a] Data obtained from Table F1, pp. 491-506, MRID 45232501. Coefficients of variance are presented in brackets. Percent of control is presented parenthetically.

TABLE 10B. Total individual activity count on PND 14 (no. of pups per count category) [a]								
Total individual activity count	Dietary concentration (ppm)							
	0	20	100	500	0	20	100	500
	Males (n = 19-20)				Females (n = 19-20)			
0-200	5	8	11	9	2	5	8	11
201-400	4	4	3	6	2	3	3	4
401-600	4	3	3	1	6	3	2	2
601-800	2	4	0	0	4	5	5	1
801-1000	3	0	2	1	5	4	2	1
>1000	1	1	1	2	1	0	0	1

[a] Data obtained from Tables F5 and F6, pp. 511-526 and 575-590, MRID 45232501.

24

TABLE 10C. Mean (±S.D.) motor activity subsession data (total number of movements) [a]					
Test Day	Block	Dietary concentration (ppm)			
		0	20	100	500
Females (n = 19-20)					
PND 14	1	35.3 ± 28.7	30.1 ± 24.8	33.6 ± 27.2	26.6 ± 21.7
	2	26.6 ± 18.3	30.6 ± 19.6	26.7 ± 26.4	24.6 ± 31.5
	3	39.2 ± 22.8	37.0 ± 24.9	23.7 ± 19.1	22.2 ± 28.2
	4	42.2 ± 26.9	32.3 ± 23.4	23.5 ± 22.2	23.3 ± 31.0
	5	39.4 ± 23.7	30.0 ± 23.3	27.3 ± 24.6	20.4 ± 26.5
	6	30.4 ± 23.0	28.9 ± 27.8	25.8 ± 21.4	19.5 ± 24.6
	7	33.7 ± 27.2	35.0 ± 23.7	21.6 ± 19.2	18.1 ± 24.0
	8	32.4 ± 28.5	31.4 ± 24.6	22.2 ± 23.8	15.6 ± 28.2
	9	37.6 ± 29.6	25.0 ± 26.6	20.9 ± 21.3	14.5 ± 21.6*
	10	36.3 ± 20.1	25.8 ± 24.6	17.4 ± 17.8*	14.3 ± 20.7**
	11	38.7 ± 26.3	19.2 ± 26.0*	16.6 ± 15.8**	7.4 ± 9.5**
	12	34.3 ± 22.6	16.2 ± 21.8*	17.6 ± 18.6*	10.1 ± 20.0**
	13	18.5 ± 19.0	23.2 ± 28.2	26.5 ± 27.1	11.4 ± 22.1
	14	19.6 ± 19.6	24.5 ± 25.2	18.8 ± 23.3	14.8 ± 21.4
	15	30.8 ± 26.9	30.2 ± 28.2	20.4 ± 24.5	13.0 ± 22.1
	16	35.6 ± 28.2	30.1 ± 28.0	18.1 ± 15.9	13.1 ± 22.5*
	17	37.6 ± 23.9	22.6 ± 23.6	23.7 ± 24.1	14.2 ± 19.4**
	18	42.8 ± 26.6	16.1 ± 22.3**	16.6 ± 20.8**	16.1 ± 21.5**
	Mean	610.7 ± 268.7	488.2 ± 303.8	401.1 ± 311.8	299.1 ± 352.1**

[a] Data obtained from Table F1, pp. 493, MRID 45232501.
Each block consists of a 5-minute period.
Significantly different from control: *$p \le 0.05$; **$p \le 0.01$.

c. **Auditory startle reflex**: Data for auditory startle habituation are summarized in Table 11. No dose- or treatment-related differences were noted between the treated and control groups on either testing day. Sporadic, incidental significant differences from the controls included increases in response magnitude by the low-dose males during block 5 on PND 23 and by the mid-dose females during block 4 on PND 61. A consistent pattern of increased response magnitudes was noted in treated females on PND 61; the interpretation of this effect is unclear.

Subsession (block) data demonstrated only minimal habituation at PND 23 (which appears to be somewhat more pronounced in control than in treated animals); habituation was evident in both sexes of all groups at PND 61 testing.

The data presentation in the report precluded a simple evaluation of the individual data for auditory startle amplitude; individual values were presented as raw data, uncorrected for baseline. Since baseline varies systematically with weight (for the startle procedure used in this study), and since body weights varied by treatment groups in the current

27

study, individual startle amplitude values cannot be directly compared across groups until they have been corrected for baseline differences. Although the mean and summary data were presented in the study report as corrected values, these individual corrected values were not provided. Future reports from this laboratory should include individual corrected values, from which the means were derived.

	Block	TABLE 11. Auditory startle habituation response magnitude (mean g ± S.D.) [a]			
		Dietary concentration (ppm)			
		0	20	100	500
Males (n = 18-20)					
PND 23	1	16.8 ± 7.4	14.9 ± 7.3	15.9 ± 7.2	14.5 ± 9.7
	2	11.9 ± 8.6	10.4 ± 4.9	12.0 ± 6.1	9.6 ± 5.9
	3	11.7 ± 9.1	10.3 ± 4.3	13.0 ± 9.6	9.1 ± 5.7
	4	11.1 ± 8.6	13.1 ± 6.0	13.2 ± 11.4	9.1 ± 6.7
	5	9.4 ± 6.5	17.2** ± 8.9	13.3 ± 7.6	9.9 ± 7.5
	Avg.	12.2 ± 7.3 [60]	13.2 ± 4.8	13.5 ± 7.7	10.5 ± 6.4 (86)
PND 61	1	98.3 ± 64.3	100.4 ± 51.7	93.2 ± 58.3	92.6 ± 50.9
	2	66.3 ± 52.9	60.1 ± 42.2	56.9 ± 38.1	63.7 ± 50.6
	3	45.9 ± 32.5	49.7 ± 30.1	45.5 ± 33.2	52.0 ± 35.7
	4	49.4 ± 37.7	39.8 ± 28.8	33.7 ± 28.6	47.2 ± 46.5
	5	38.3 ± 27.6	35.6 ± 29.2	32.6 ± 31.6	39.6 ± 34.6
	Avg.	59.6 ± 36.7 [62]	57.1 ± 31.8	52.4 ± 32.5	59.0 ± 39.3 (99)
Females (n = 19-20)					
PND 23	1	16.5 ± 10.3	13.7 ± 4.9	14.5 ± 7.6	14.4 ± 7.2
	2	12.0 ± 6.8	10.5 ± 5.4	10.7 ± 7.5	11.4 ± 4.5
	3	10.5 ± 5.2	11.9 ± 6.6	9.3 ± 6.0	10.0 ± 6.4
	4	10.1 ± 6.0	12.7 ± 7.0	9.8 ± 6.7	10.3 ± 5.5
	5	9.0 ± 5.3	14.5 ± 10.1	11.1 ± 8.8	12.3 ± 6.0
	Avg.	11.6 ± 5.6	12.7 ± 5.7	11.1 ± 6.6	11.7 ± 4.1 (101)
PND 61	1	49.0 ± 31.0 [48]	56.0 ± 51.8	65.0 ± 48.5	51.5 ± 34.3
	2	23.8 ± 15.8	46.0 ± 69.3	37.5 ± 28.6	30.3 ± 18.1
	3	18.7 ± 14.3	27.9 ± 30.7	32.8 ± 24.9	30.5 ± 21.5
	4	15.7 ± 8.9	19.5 ± 14.1	29.4* ± 23.2	24.4 ± 15.8
	5	14.3 ± 10.2	19.7 ± 23.7	25.8 ± 19.1	16.6 ± 13.9
	Avg.	24.3 ± 10.9 [45]	33.8 ± 36.5	38.1 ± 25.5	30.7 ± 18.0 (126)

[a]Data obtained from Table F2, pp. 507-508, MRID 45232501. Coefficients of variance are presented in brackets. Percent of control is presented parenthetically.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

d. Learning and memory testing:

Passive avoidance: Data summarizing performance in the passive avoidance test are presented in Table 12. No treatment-related effects on the acquisition and memory of the avoidance task were reported. However, a number of problems were noted, including the following: 1) The report included data for only the first two trials. Data (mean or individual) for subsequent trials

were not included in the report; the limited data presented do not allow evaluation of the complete learning curve across trials, or other aspects of performance (for individuals or across the groups) 2) The data presented did not reliably demonstrate that the rats were learning and retaining the acquisition task. In particular, for high dose females there does not appear to be a reliable difference between session 1 trial 1 and session 2 trial 1 latencies (i.e., retention was not demonstrated, for example in the control female data presented below). Although simple inspection of the mean values does demonstrate a tendency toward increased latency from trial 1 to trial 2 (session 1), the variability is large. Many of the rats had similar latencies in the first and second trials of the first session, and their latency scores for the second session did not demonstrate any clear relationship to their scores from the first session. Availability of data from remaining learning trials might address some of these issues. 3) Three animals (one mid-dose male, one high-dose male, and one high-dose female) remained in the lighted portion of the apparatus for 60 seconds on both the first and second trials of session 1 (i.e., they were scored as having correctly learned the task although they had never experienced the aversive stimulus), but then were found dead on PND 26 (male pups) or 24 (female pup); their failure to move into the dark chamber on the first trial, especially when taken together with their subsequent deaths, suggests these animals were not capable of performing this task. These animals should not have been included in the mean calculations. 4) The submitted positive control data did not include a study demonstrating the capability of the performing laboratory in detecting treatment-related effects on acquisition and memory using a passive avoidance paradigm. The absence of positive control data, when combined with the data presentation and variance problems noted above, raise questions about whether this task satisfies the guideline recommendations for learning and memory evaluation.

TABLE 12. Passive avoidance performance at PND 24 [a]					
Test Day/Parameter		Dietary concentration (ppm)			
		0	20	100	500
Males (n = 19-20)					
Session 1	Trials to criterion	5.2 ± 2.1	6.0 ± 4.1	4.6 ± 1.2	4.3 ± 1.0
	Latency trial 1 (sec)	9.4 ± 6.1	8.4 ± 5.9	12.2 ± 12.9	11.9 ± 12.2
	Latency trial 2 (sec)	27.8 ± 20.8	28.2 ± 18.7	35.2 ± 21.6	36.0 ± 18.8
	Failed to learn	0	2	0	0
Session 2	Trials to criterion	3.2 ± 2.9	3.4* ± 0.9	2.9 ± 0.8	3.0 ± 1.1
	Latency trial 1 (sec.)	38.3 ± 22.5	21.0 ± 21.0	29.2 ± 24.9	27.8 ± 21.9
Females (n = 19-20)					
Session 1	Trials to criterion	5.0 ± 1.5	4.3 ± 1.0	5.0 ± 1.4	4.8 ± 1.6
	Latency trial 1 (sec)	11.5 ± 9.7	6.6 ± 5.0	7.0 ± 3.8	9.5 ± 5.6
	Latency trial 2 (sec)	24.9 ± 21.0	27.2 ± 21.0	17.6 ± 14.0	28.9 ± 19.5
	Failed to learn	0	0	0	0
Session 2	Trials to criterion	3.8 ± 3.0	3.1 ± 0.7	3.2 ± 1.2	3.5 ± 1.7
	Latency trial 1 (sec.)	19.9 ± 19.4	22.3 ± 22.9	21.0 ± 19.5	11.2 ± 13.5

[a] Data obtained from Table E1, p. 462, MRID 45232501.
Significantly different from control: *$p \leq 0.05$.

29

Water maze: Data for males and females are summarized in Table 13. No differences from control were observed among treated groups in water maze testing of adult offspring. However, many of the data presentation and interpretation problems noted above with respect to the passive avoidance task are also apparent in the reported data for this task; in particular the limited data presentation in the study, large variance, failure of presented data to demonstrate learning (during session 1) or retention (during session 2) for control rats, and lack of positive control data for the water maze task. In addition, because data for session 1/trial 1 (latency or errors) was not included in the study report, acquisition could not be evaluated. As noted above, these limitations raise questions about adequate evaluation of learning and memory in this study.

TABLE 13. Water maze performance [a]				
Test Day/Parameter		**Dietary concentration (ppm)**		
		0	**20**	**100**	**500**
Males (n = 19-20)					
Session 1	Trials to criterion	9.7 ± 2.5	8.5 ± 2.1	9.2 ± 2.5	9.5 ± 3.6
	Errors per trial	0.41 ± 0.15	0.40 ± 0.20	0.46 ± 0.25	0.44 ± 0.25
	Latency trial 2 (sec)	12.2 ± 4.8	13.2 ± 6.5	15.5 ± 11.5	12.8 ± 6.7
	Failed to learn	0	0	0	2
Session 2	Trials to criterion	6.5 ± 1.7	8.0 ± 3.7	6.1 ± 2.2	8.4 ± 3.9
	Errors per trial	0.14 ± 0.16	0.18 ± 0.25	0.13 ± 0.21	0.19 ± 0.22
	Latency trial 1 (sec)	14.3 ± 10.7	9.3 ± 6.3	9.7 ± 4.7	10.8 ± 4.8
Females (n = 19-20)					
Session 1	Trials to criterion	8.6 ± 3.1	10.0 ± 2.8	10.1 ± 3.1	10.7 ± 3.1
	Errors per trial	0.35 ± 0.16	0.36 ± 0.13	0.47 ± 0.29	0.45 ± 0.23
	Latency trial 2 (sec)	13.2 ± 5.9	11.6 ± 4.2	17.6 ± 9.3	15.0 ± 11.4
	Failed to learn	2	0	1	2
Session 2	Trials to criterion	7.0 ± 2.9	7.6 ± 2.6	8.8 ± 3.2	7.2 ± 2.8
	Errors per trial	0.12 ± 0.14	0.25 ± 0.21	0.28 ± 0.27	0.11 ± 0.15
	Latency trial 1 (sec.)	14.6 ± 10.6	11.2 ± 8.4	13.9 ± 13.5	9.1 ± 5.3

[a] Data obtained from Table E2, p. 463, MRID 45232501.

6. Postmortem results:

a. **Gross necropsy:** No treatment-related gross lesions were observed in the offspring at any scheduled sacrifice.

b. **Organ weights:** Mean absolute and relative organ weight data are summarized in Table 14. On PND 12, mean terminal body weights of the high-dose males and females were 83.3% (p ≤ 0.01) and 87.6% (n.s.), respectively, of the control levels. Consequently, mean brain-to-body weight ratios were increased (p ≤ 0.05 or 0.01) for high-dose males and

females compared with the controls. In addition, mean absolute liver weights of the mid- and high-dose males were significantly ($p \leq 0.05$ or 0.01) less than that of the control; relative liver weight values were comparable to control. No other differences in absolute or relative organ weights were found on PND 12.

For pups sacrificed on PND 22, mean body weights of all treated males and females were slightly less than those of the controls, but the effect was not dose-related. No differences from control in liver or thyroid weights were seen.

At study termination (PND 72-76), the mean body weight of the high-dose males was 90.6% (n.s.) of the control level. The mean body weight of the high-dose females and the mean brain weights of males and females in the treated groups were similar to those of the controls.

31

TABLE 14. Terminal body weight and organ weight data (mean ± SD) in offspring [a]				
Parameter	**Dietary concentration (ppm)**			
	0	**20**	**100**	**500**
Males				
Day 12 (n = 19-20)				
Terminal body weight (g)	20.4 ± 2.9	18.9 ± 3.2	18.8 ± 3.0	17.0** ± 4.7
Brain wt: absolute (g)	1.133 ± 0.102	1.124 ± 0.152	1.093 = 0.139	1.054 ± 0.172
relative to body weight (%)	5.618 ± 0.562	6.017 ± 0.523	5.867 ± 0.529	6.439* ± 1.060
Liver wt: absolute (g)	0.74 ± 0.16	0.68 ± 0.11	0.63* ± 0.14	0.58** ± 0.13
relative to body weight (%)	3.635 ± 0.689	3.658 ± 0.737	3.403 ± 0.700	3.516 ± 0.606
relative to brain weight (%)	65.55 ± 14.96	60.71 ± 10.01	59.01 ± 14.08	55.35 ± 9.25
Thyroid wt: absolute (g)	0.005 ± 0.002	0.005 ± 0.002	0.004 ± 0.001	0.004 ± 0.001
relative to body wt (%x1000)	24.637 ± 7.249	24.593 ± 9.566	24.232 ± 9.259	23.386 ± 5.227
relative to brain weight (%)	0.44 ± 0.13	0.41 ± 0.16	0.42 ± 0.16	0.37 ± 0.11
Day 22 (n = 15-20)				
Terminal body weight (g)	46.7 ± 4.5	42.3 ± 4.8	43.8 ± 4.3	43.5 ± 8.7
Liver wt: absolute (g)	1.97 ± 0.26	1.86 ± 0.30	1.90 ± 0.25	1.88 ± 0.44
relative to body weight (%)	4.220 ± 0.345	4.397 ± 0.462	4.342 ± 0.386	4.302 ± 0.396
Thyroid wt: absolute (g)	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001
relative to body wt (%x1000)	14.750 ± 1.534	15.586 ± 2.510	16.525 ± 2.742	16.159 ± 3.269
Day 72-76 (n = 6)				
Terminal body weight (g)	445.5 ± 45.3	438.2 ± 28.6	428.3 ± 40.3	403.7 ± 25.0
Brain wt: absolute (g)	2.13 ± 0.09	2.04 ± 0.11	2.11 ± 0.10	2.05 ± 0.09
relative to body weight (%)	0.480 ± 0.048	0.467 ± 0.037	0.495 ± 0.031	0.508 ± 0.016
Females				
Day 12 (n = 19-20)				
Terminal body weight (g)	19.3 ± 3.1	18.2 ± 3.1	18.0 ± 3.4	16.9 ± 3.9
Brain wt: absolute (g)	1.10 ± 0.10	1.07 ± 0.12	1.06 ± 0.14	1.05 ± 0.17
relative to body weight (%)	5.798 ± 0.642	5.996 ± 0.591	5.919 ± 0.546	6.389** ± 0.828
Liver wt: absolute (g)	0.71 ± 0.14	0.63 ± 0.10	0.66 ± 0.17	0.61 ± 0.14
relative to body weight (%)	3.718 ± 0.642	3.552 ± 0.631	3.684 ± 0.729	3.650 ± 0.579
relative to brain weight (%)	64.82 ± 13.15	59.33 ± 9.37	62.88 ± 14.30	57.72 ± 10.37
Thyroid wt: absolute (g)	0.003 ± 0.001	0.004 ± 0.001	0.003 ± 0.001	0.003 ± 0.001
relative to body wt (%x1000)	17.984 ± 3.053	19.460 ± 4.068	18.888 = 4.255	17.319 ± 4.388
relative to brain weight (%)	0.31 ± 0.06	0.33 ± 0.08	0.32 ± 0.07	0.27 ± 0.07
Day 22 (n = 18-20)				
Terminal body weight (g)	45.1 ± 7.5	41.8* ± 3.9	40.8* ± 5.6	40.8 ± 9.0
Liver wt: absolute (g)	1.92 ± 0.39	1.86 ± 0.26	1.84 ± 0.32	1.74 ± 0.47
relative to body weight (%)	4.236 ± 0.371	4.445 ± 0.348	4.505 ± 0.578	4.244 ± 0.426
Thyroid wt: absolute (g)	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001
relative to body wt (%x1000)	15.855 ± 1.758	17.049 ± 2.829	17.024 ± 2.433	17.538 ± 3.885
Day 72-76 (n = 6)				
Terminal body weight (g)	237.8 ± 25.8	239.8 ± 20.9	249.5 ± 20.9	234.3 ± 21.8
Brain wt: absolute (g)	1.93 ± 0.08	1.92 ± 0.09	1.90 ± 0.07	1.92 ± 0.09
relative to body weight (%)	0.818 ± 0.069	0.807 ± 0.100	0.763 ± 0.058	0.823 ± 0.052

[a] Data obtained from Tables D3-D8, pp. 431-436, Tables G3 and G4, pp. 666-667, and Tables H3-H6, pp. 681-684, MRID 45232501.

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

c. **Qualitative histopathology:** No dose- or treatment-related microscopic lesions were found in the liver or thyroid/parathyroid in any animal of either sex sacrificed on PND 12 or 22. Common findings in treated and control animals included extramedullary hematopoiesis in the liver and an occasional cystic ultimobranchial body in the thyroid gland.

Neuropathology findings (central and peripheral nervous system) are presented in Table 15. No treatment-related effects were reported for male or female offspring at postnatal days 12 or 72-76.

TABLE 15. Qualitative neuropathology incidence data in offspring [a]					
Tissue/Finding	Grade	Dietary concentration (ppm)			
		0	500	0	500
		Males		Females	
Day 12					
Medulla oblongata					
Ossification of choroid plexus	Moderate	1	0	0	0
Cerebellar cortex					
Edema/vacuolation	Mild	0	0	1	1
Cerebellar nuclei					
Edema/vacuolation	Mild	0	0	1	0
Cerebellar white matter					
Edema/vacuolation	Mild	0	0	2	1
Day 72-76					
Spinal cord, thoracic					
Myelin sheath swelling	Minimal	2	1	0	0
Spinal cord, lumbar					
Myelin sheath swelling	Minimal	2	1	0	0
Axonal swelling	Minimal	0	0	0	1
Spinal nerve roots					
Myelin sheath swelling	Minimal	2	1	0	0
Demyelination	Minimal	0	1	0	0
Gasserian ganglia					
Neuronal vacuolation	Mild	1	0	0	0
Sciatic nerve					
Myelin sheath swelling	Minimal	1	0	0	0

[a] Data obtained from Appendix K, Tables 3 and 4, pp. 775-784, and Appendix L, Tables 3 and 4, pp. 818-829, MRID 45232501.

c. **Quantitative histopathology (brain morphometry):** Linear measurements of the brains of PND 12 and 72-77 control and high-dose animals and of PND 72-77 intermediate-dose females are presented in Table 16.

33

Parameter	Dietary concentration (ppm)				
	0	500	0	100	500
	Males		Females		
Day 12					
Brain weight (g)	1.148 ± 0.094	1.087 ± 0.172	1.1583 ± 0.0875	N/A	1.118 ± 0.104
Cerebrum: anterior to posterior (mm)	10.50 ± 0.55	9.83 ± 0.98	10.83 ± 0.75	N/A	10.67 ± 0.82
Cerebellum: anterior to posterior (mm)	6.00 ± 0.63	5.67 ± 0.52	6.00 ± 0.63	N/A	6.33 ± 0.52
Frontal cortex (μm)	1604.0 ± 122.8	1508.0 ± 112.0	1540.0 ± 78.0	N/A	1604.0 ± 135.3
Parietal cortex (μm)	1620.0 ± 82.8	1508.0 ± 137.8	1592.0 ± 65.6	N/A	1608.0 ± 98.4
Caudate putamen (μm)	2792.0 ± 188.2	2592.0 ± 322.7	2752.0 ± 112.2	N/A	2664.0 ± 162.8
Corpus callosum (μm)	291.2 ± 78.2	248.0 ± 42.6	248.0 ± 21.5	N/A	281.7 ± 43.2
Dentate gyrus (μm)	1124.0 ± 63.3	1068.0 ± 124.9	1080.0 ± 48.0	N/A	1068.0 ± 56.3
Cerebellum (μm)	3328.0 ± 255.2	3232.0 ± 514.9	3609.6 ± 331.5	N/A	3544 ± 175.5
External germinal layer (μm)	37.8 ± 2.7	37.8 ± 2.7	41.5 ± 4.2	N/A	42.3 ± 4.8
Day 72-76					
Brain weight (g)	2.127 ± 9.093	2.050 ± 0.086	1.933 ± 0.085	1.898 ± 0.074	1.923 ± 0.088
Cerebrum: anterior to posterior (mm)	14.08 ± 0.20	14.25 ± 0.42	13.83 ± 0.41	14.33 ± 1.63	13.83 ± 0.41
Cerebellum: anterior to posterior (mm)	7.00 ± 0.32	7.08 ± 0.20	6.83 ± 0.41	6.83 ± 0.75	6.83 ± 0.41
Frontal cortex (μm)	1776.0 ± 110.5	1764.0 ± 102.7	1640.0 ± 78.4	NR	1548.0 ± 92.0
Parietal cortex (μm)	1868 ± 105.6	1852.0 ± 94.1	1704.0 ± 48.0	NR	1652.0 ± 44.0
Caudate putamen - diagonal (μm)	3352 ± 239.8	3288.0 ± 134.9	3204 ± 116.96	2868** ± 176.7 (90)	2900* ± 256.44 (91)
Caudate putamen - transverse (μm)	N/A	N/A	2832 ± 98.59	2688** ± 78.23 (95)	2590** ± 96.12 (91)
Corpus callosum (μm)	284.8 ± 36.3	249.7 ± 37.9	252.5 ± 46.6	NR	248.0 ± 17.9
Dentate gyrus (μm)	1616.0 ± 67.3	1556.0 ± 65.1	1468.0 ± 51.3	NR	1432.0 ± 65.6
Cerebellum (μm)	4600.0 ± 195.4	4648.0 ± 302.7	4512.0 ± 163.5	NR	4360.0 ± 285.5

TABLE 16. Mean (±SD) morphometric data in offspring [a]

[a]Data obtained from Appendix K, Tables 1 and 2, pp. 771-774, and Appendix L, Tables 1 and 2, pp. 812-817, MRID 45232501. Percent of control is presented parenthetically.
N/A = not applicable; this measurement was not taken.
NR = not reported
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

No statistical differences were found in microscopic measurements between the control and high-dose animals on PND 12. However, on PND 12, most of the parameters for the treated males were slightly less than those of the controls. It is noted that the transverse measurements of the caudate putamen were not evaluated for the males (no explanation was provided).

For adult F1 males, no differences were observed between the control and high-dose groups for any parameter. For mid- and high-dose adult F_1 females, both linear measurements of the striatum (diagonal and transverse) were significantly (p≤0.05 or

0.01) less than those of the controls (data for the mid-dose group were not included in the report). Data from the morphometric evaluation of the mid-dose males was considered unreliable by the study pathologist, and was in large part not included in the study report. The study pathologist concluded that the alterations in female caudate putamen measurements were not treatment-related. However, it is noted by Agency reviewers that 1) the decreases in transverse caudate putamen measurements for F1 adult females are dose-related, 2) although the decreases in diagonal caudate putamen measurements are not clearly dose-related, the variation in this measurement was more than double that of the transverse measures for F1 adult females, and 3) intermediate-dose data were not reported for adult F1 males, nor were frontal cortex, parietal cortex, corpus callosum, dentate gyrus, or cerebellum measures for F1 adult females at the intermediate dose, thereby providing a very incomplete picture of brain morphometry at that dose. Historical control morphometry data included in the study report (MRID 45796117, p. 952) cannot be used to clarify this issue, since no information is provided regarding the source of these data or the methods used for fixation, embedding, or sectioning the brain tissues; additionally, separate measurements for diagonal and transverse sections of the caudate putamen are not included. According to the study report, the study author and pathologist had no confidence in the veracity of the intermediate dose striatal measures; in that case, it is unclear how a definitive no-adverse-effect-level can be determined for observed decreases in effects on caudate putamen measurements. Based upon information provided in the study report, it is unlikely that further histopathological evaluation of brain tissues would resolve this uncertainty. However, these data should be submitted for Agency evaluation.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The author concluded that maternal toxicity was observed at 100 and 500 ppm as decreases in body weight, weight gain (high-dose only), and food consumption during gestation. Offspring toxicity was evident as decreases in body weights, body weight gains, and food consumption as well as developmental delays in pups from dams administered 100 and 500 ppm. The author determined that the NOEL for both maternal and offspring toxicity was 20 ppm, and that the test article did not cause developmental neurotoxicity.

B. REVIEWER COMMENTS:

Maternal toxicity:

Agency reviewers disagreed with the study author's opinion that body weight gain deficits were not noted at the mid-dose, since there was a significant, dose-related reduction in maternal body weight gain at GD 6-9. However, it is noted that the effects on maternal body weight, body weight gain, and food consumption were transient, and occurred immediately after initiation of dietary treatment. This pattern of response is suggestive of palatability problems. In support of that interpretation, both the mid- and high-dose groups showed slightly or significantly greater absolute and relative food consumption during GD 12-15, resulting in slightly greater mean body weight gains by these groups for this interval which suggests an adaptation to the treated feed.

35

Therefore, while recognizing that the evidence of maternal systemic toxicity in this study was related to treatment, the transient decreases in body weight, body weight gain, and food consumption at the mid- and high-doses were not considered to be adverse.

Summary of maternal toxicity: All dams survived to scheduled termination and no clinical signs of toxicity were observed. During gestation, transient decreases in maternal body weight, body weight gain, and food consumption were observed in the mid- and high-dose groups (100 and 500 ppm) following the initiation of dietary treatment. Absolute body weights for the mid- and high-dose groups were significantly less than those of the control group on gestation days (GD) 8-9 (96% of control) and GD 8-12 (93-96% of control), respectively. Significant reductions in body weight gains were observed for the mid-dose group during GD 6-9 (67% of control) and for the high-dose group for the intervals of GD 6-9 (37% of control) and 6-21 (86% of control). Decreases in body weight gains correlated with significantly reduced food consumption as compared to controls during GD 6-9 for the mid-dose group (84% of control) and GD 6-12 for the high-dose group (72-84% of control). Relative food consumption was also significantly decreased for the mid-dose group (GD 6-9) and the high-dose group (GD 6-12). There were no treatment-related effects on maternal body weight or food consumption during the lactation period. Reproductive performance was not affected by treatment. In light of their small magnitude, short duration, and correlation with decreased food consumption (indicative of a possible palatability problem), the decreases in maternal body weight and body weight gain during early gestation were not considered adverse. **The maternal LOAEL for flufenacet in rats is not determined. The maternal NOAEL is 500 ppm (40.8 mg/kg/day).**

Offspring toxicity:

Agency reviewers disagreed with the study author on the NOAEL for offspring effects. Significant decreases in pre- and postweaning body weight and/or body weight gain for offspring at the lowest dose tested were ignored by the study author in establishing the NOAEL for the study, but are clearly treatment-related and cannot be discounted.

There is also a difference in interpretation of the decreased motor activity counts observed at the mid- and high-dose levels for the PND 14 female pups. The study author did not judge these findings to be biologically significant. However, as addressed earlier in this DER, Agency reviewers believe that these findings are treatment-related, supporting this position with additional analysis of the subsession and individual subject data. Additionally, these findings can be correlated to developmental delays at the age of assessment (specifically, body weight deficits and delays in eye opening).

The assessment of thyroid hormone measures in this study are limited in scope by the route of administration (i.e., maternal dietary exposure only; unknown actual doses to either dams or offspring). They do not provide sufficient information to quantitatively evaluate a comparison of the response of adults and offspring to thyroid perturbations at similar doses.

Summary of offspring toxicity: There were no effects of treatment on offspring survival. Offspring development and growth were affected in all treated groups. Significant decreases

34

in body weight and body weight gain were observed in all treated groups. These weight deficits were observed starting at PND 5 for high-dose pups, and at PND 12 for low- and mid-dose pups. Postweaning recovery to control body weight levels was observed in low-dose offspring and in mid-dose females. Other evidence of delayed offspring development at the mid- and high doses included significant delays in the age at eye opening and in the age of preputial separation in males.

Serum T_3 and T_4 measures of blood collected at PND 12 and 22 were not affected by treatment. No treatment-related effects on liver or thyroid/parathyroid weights or histopathology were observed in the offspring.

Neurobehavioral assessment of the offspring revealed no treatment-related effects on autonomic function, auditory startle habituation, or learning and memory testing (passive avoidance and water maze). However, questions regarding the reliability of some of these assessments (in particular the learning and memory assessments) were discussed above. Treatment-related decreases in motor activity counts were observed in PND 14 female pups at the mid- and high-dose. Neuropathological evaluation revealed significant decreases in caudate putamen measurements for the adult female offspring at the high dose. Subsequent analysis of the mid-dose brains confirmed a similar significant response; low-dose brains were not evaluated.

The offspring LOAEL is 20 ppm (1.7 mg/kg/day), based on decreased preweaning body weight and body weight gain. The offspring NOAEL is not determined. In addition to the decreased body weight and body weight gain observed at the LOAEL, significant treatment-related findings at 100 ppm (8.3 mg/kg/day) and 500 ppm (40.8 mg/kg/day) include delayed eye opening, delayed preputial separation in males, decreased motor activity counts for PND 14 females, and decreased caudate putamen measurements in the brains of adult female offspring. The NOAEL for these additional mid- and high-dose offspring effects is 20 ppm (1.7 mg/kg/day) with the exception of the decreased cauate putamen measurements, for which a NOAEL can not be established (due to lack of morphometry data for 20 ppm offspring).

C. STUDY DEFICIENCIES:

— Concentration analyses were not reported for the dietary mixtures used in this study. Analytical data from a reproduction study (reported in 1995) cannot be used to support the adequacy of the formulations prepared for this developmental neurotoxicity study (which was conducted in 1998).
— No functional behavioral evaluations, not even detailed clinical observations, were performed on preweaning offspring. For postweaning offspring, detailed clinical observations were conducted. However, the procedures used for these evaluations were not described, including whether the same technicians were used throughout testing, where the testing was done (including whether the animals were removed from the cage), what the environmental conditions were (e.g., noise level, etc.), whether scoring criteria were used for the measured parameters, or the duration of the observation period for open field observations.

37

FLUFENACET (THIAFLUAMIDE)/121903

— A number of problems and deficiencies were noted for the passive avoidance and water maze test data (see DER text for specifics). Individual trial data should be submitted, as well as summary data for trials not previously reported, in order to facilitate the interpretation of the test results. Submitted data should include Session 1/Trial 1 latency data for water maze testing.

— Measurements were made bilaterally for a number of areas of the brain in both PND 12 and adult offspring, but only the mean values were reported. Values for linear brain measurements that were recorded, but not included in the current report, should be submitted.

— Following the initial review of morphometry data, a determination was made to evaluate the mid-dose brains. The pathologist and study director expressed a lack of confidence in the quality of the resulting data and reported only selected measurements. This is not acceptable, in accordance with Good Laboratory Practice Regulations. All data for evaluated parameters should be submitted. Additionally, an explanation should be provided regarding whether further evaluation of the low-dose brains is feasible at this point.

— A minor discrepancy exists in the age at sacrifice of the adult F_1 animals: individual data in Appendix G lists day of necropsy as PND 72-76, however, the neuropathology report in Appendix L states that animals were 83 days old. This discrepancy should be explained.

— Acceptable positive control data were not provided.

Attachment 1 – Positive Control Data

Positive control data were provided in MRID 45796117. These data had been previously submitted to the Agency in MRID 45074301, and all pages of the volume submitted tosupport the current DNT study inappropriately retained the study header information, Appendix identification, and pagination from the DNT study with the previous test substance.

Most of the information in this volume consisted of summaries of studies performed by scientists currently or formerly affiliated with the study laboratory. Some of the studies were performed at the study laboratory, others at institutions with which the scientists were previously affiliated.

The summarized information included a variety of studies (summarized briefly below), largely relevant to the development of the procedures used in the current developmental neurotoxicity and adult neurotoxicity study protocols at the study laboratory. However, apart from specific exceptions described below, the submitted information is not fully adequate to support the sensitivity of many of the procedures used in the current study.

Current Agency guideline recommendations for positive control data are as follows:
Appropriate, adequate positive control data from the laboratories that performed the Developmental Neurotoxicity studies should be provided to the Agency at the time of study submission. These positive control data should demonstrate the sensitivity of the procedures used, including the ability to detect both increases and decreases in parameters measured, as appropriate. While the positive control studies do not need to be performed using prenatal exposures, the laboratory must demonstrate competence in the evaluation of effects in neonatal animals perinatally exposed to chemicals and establish test norms for all critical endpoints, and for appropriate age groups. The positive control data should be derived from relatively recent studies, that is, studies that were performed in the same laboratory within the past few years, utilizing (to the greatest extent possible) the staff and equipment that will be used in conducting the current studies.

Based on a review of the submitted information, most of the submitted studies were performed outside of the recommended time frame, and laboratory personnel varied from those involved in the current study. It is unclear whether test procedures were the same as those used in the current study, and not all procedures were evaluated following exposure to neurotoxic substances. Insufficient information was included for most studies; for example, individual animal data were rarely included and detailed procedural information was often not provided.

Since treatment-related effects were seen at all doses evaluated in the current study, we will not require submission of additional positive control data prior to acceptance of that study. It is noted, however, that insufficiently sensitive procedures could lead to a failure to detect effects on some parameters (for example, cognitive or motor activity testing) or a failure to detect effects at low doses.

Based on an evaluation of the submitted positive control data, according to current recommendations, additional data are needed to adequately validate the sensitivity and reliability

39

of the procedures listed below, as currently used in the testing laboratory. The additional positive control data should be generated using current personnel and equipment, with the same procedures as those used in the study, and using the same strain of rat. Complete study reports should be submitted, including individual animal data.

Procedures lacking appropriate positive control data:
    1) Motor activity evaluation, all time points;
    2) Learning and memory procedures, both time points;
    3) Auditory startle habituation, both time points;
    4) Neuropathology evaluations:
        -qualitative evaluations in treated pups
        -morphometric evaluations in adults (late time point).

As noted below, the submitted positive control data for pup morphometric evaluations was considered adequate (Study #12), but the submission for adult qualitative evaluations was incomplete, since the data tables were not provided. The adequacy of the data supporting adult qualitative evaluations cannot be determined in the absence of the data tables; those data should be submitted.

Summary of submitted studies

Each of the submitted studies, along with a brief description, is listed below. Studies are grouped according to the type of data included.

FOB validation studies

1. Parker, R.M. (1999) Neurotoxicity evaluation of positive control substances in Crl:CD®BR VAF/Plus® Rats. Argus Research Laboratories, Horsham, PA. Laboratory protocol no. 012-075. August 6, 1999, Unpublished study. [incorrectly identified as Appendix O, Section 10, pp. 954-1131]

   The study evaluated FOB performance using several known neurotoxic chemicals (acrylamide, Trimethyl tin, MK-801, Carbaryl, and DDT). Evaluations were made in 59-day old rats. This appears to be a GLP study, useful in validating FOB evaluations in adult rats. However, it was not possible to fully evaluate the adequacy of these data, because the portion of the report containing the individual animal data was excluded from the submission (study report pp. 143-229). Since full FOB evaluations were not performed in the current study, these data are not considered critical in support of this study.

2. [author not provided] (1992) Neurotoxicity evaluation of DDT in Crl:CD®BR VAF/Plus® Rats. Argus Research Laboratories, Horsham, PA. Laboratory protocol no. 012-015. [incorrectly identified as Appendix O, Section 11, pp. 1133-1141]

40

This study was performed in 1992 at Argus Laboratories, and evaluated 50/51 day old rats on the FOB. As above, these data are relevant to FOB evaluations performed in adult rats, which were not performed in the current study.

FOB and motor activity in adult rats

3. a) Lochry, E.A., J.A. Foss, and M.S. Christian (1990). Validation of a functional observational battery and motor activity measure using positive test substances. Argus Research Laboratories, Poster presented at the Annual Meeting of the American College of Toxicology, Orlando, Florida, October 1990.
    b) Foss, J.A. (1992) Argus Research Laboratories, Horsham, PA. Laboratory protocol no. 012-016. [incorrectly identified as Appendix O, Section 12, pp. 1142-1174]

This information consists of a poster copy, with associated individual and/or summary data, and a protocol and data for a motor activity study that was conducted two years after the meeting (and therefore are not the source of the motor activity data that are summarized in the poster). The poster describes a study that was performed on adult rats (65 days old at arrival in the laboratory), and assessed performance of FOB parameters following administration of DDT, physostigmine, and acrylamide and motor activity measures following administration of chlorpromazine and d-amphetamine. The later motor activity study also assessed effects in adult rats following chlorpromazine and d-amphetamine administration. The data demonstrated decreased habituation following d-amphetamine administration.

4. Foss, J.A. (1991) Neurotoxicity evaluation of positive control substances in Crl:CD®BR VAF/Plus® Rats. Argus Research Laboratories, Horsham, PA. Laboratory protocol no. 012-014. [incorrectly identified as Appendix O, Section 13, pp. 1176-1242]

This submission consisted of summary data only, concerning FOB and motor activity testing using acrylamide and carbaryl.

Studies including assessments in developing animals

5. Foss, J.A. and E.A. Lochry. (1991) The assessment of motor activity in neonatal and adult rodents using passive infrared sensors. Argus Laboratories, Poster presented at the Annual Meeting of the American College of Toxicology, Savannah, Georgia, October, 1991. [incorrectly identified as Appendix O, Section 14, pp. 1243-1250]

This section consists of a poster copy. Habituation (for motor activity) was evaluated in adult rats and mice, and in neonatal rats. Several test substances were evaluated for adult rats (including acrylamide, IDPN, DDT, and triadiamefon), but neonates were untreated, and evaluated on several different days (13, 17, 21, and 58/59). This study used passive infrared sensors to monitor motor activity of untreated adult rats, untreated adult mice, and neonatal rats on postnatal days 13, 17, 21, and 58-59. The positive control substances d-amphetamine and chlorpromazine were evaluated in rats at approximately postnatal day 60, and the positive

control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon were evaluated in adult rats. Test sessions with positive control substances were 90-115 minutes in duration and comprised of 5-minute blocks. Summary data only were presented, and it was not clear whether significant differences were detected between days for the neonates (the poster stated only that differences were detected across time within a session).

6. [No author provided] (1992) Neurotoxicity evaluation of positive control substances in Crl:CD®BR VAF/Plus® Rats. [Performing laboratory not indicated] Laboratory protocol no. 012-058. [incorrectly identified as Appendix O, Section 15, pp. 1251-1289]

   The submitted study evaluated motor activity, auditory startle, and neuropathology following treatment with acrylamide, amphetamine, TMT, and MK-801; only the motor activity data for acrylamide and amphetamine were submitted. The date of the study and personnel involved were not listed. Age of tested animals was not stated, although the weights (approximately 430 g for males and 250 g for females, pre-dosing) would indicate that adult rats were used. Motor activity data were presented as means, following treatment with acrylamide [45 mg/kg, for a maximum of 10 days], or amphetamine [0.75 mg/kg]. Testing was conducted in stainless-steel wire-bottomed cages, using passive infrared sensors; testing sessions were 90-minutes in duration, with data tabulated for each 5 minute interval. Activity levels were decreased following acrylamide treatment, and increased following amphetamine treatment.

7. Lochry, E.A. and E.P. Riley. (1980) Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. *Neurobehavioral Toxicology* 2:107-115. [incorrectly identified as Appendix O, Section 16, pp. 1291-1299]

   This was a published study, performed at the State University of New York at Albany, evaluating performance in passive avoidance and T-maze following prenatal exposure to alcohol.

8. Lochry, E.A., J.A. Foss, and M.S. Christian. (1990) Learning and retention paradigms in developmental neurotoxicity test batteries: passive avoidance and water maze. Argus Research Laboratories, Poster presented at the 18th European Teratology Society Conference, Edinburgh, Scotland, September 1990. [incorrectly identified as Appendix O, Section 17, pp. 1300-1305]

   The submitted information consists of a copy of a poster, presenting information on the performance of weanling rats in passive avoidance and adult rats in a water maze. This was a collection of historical control data (performance of untreated rats) from passive avoidance and water maze testing conducted in 1988-1989. The submission includes several tables of control data, with statistical information regarding the variance of results. Minimal procedural information was provided.

9. Foss, J.A., E.A. Lochry, and A.M. Hoberman. (1990) Automated monitoring systems for motor activity and auditory startle applicable for both developmental and adult neurotoxicity studies. Argus Research Laboratories, poster presented at the 8th International

Neurotoxicology Conference, Little Rock, AK, October 1990. [incorrectly identified as Appendix O, Section 18, pp. 1307-1320]

The submitted information consists of a copy of a poster (summary data only). Motor activity was evaluated on days 13, 17, 21, and 60 using 90 minute sessions; only untreated animals were evaluated. Apparently statistical evaluation was used to confirm habituation within sessions, no evaluation was made for differences across days. Similarly, auditory startle habituation was evaluated in untreated animals, on days 22 and 60. Habituation was demonstrated on day 60 for females, on both days for males. Minimal procedural information was included.

10. Foss, J.A. and E.P. Riley. (1989) Elicitation and modification of the acoustic startle reflex in animals prenatally exposed to cocaine. *Neurotoxicology and Teratology* 13:541-546. [incorrectly identified as Appendix O, Section 19, pp. 1321-1327]

The submitted information consists of a published article, reporting a study performed at SUNY-Albany. The potential for changes in the acoustic startle reflex was evaluated in adult rats following prenatal exposure to cocaine; no effects from exposure to test substance were demonstrated. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study.

11. Lochry, E.A., Hoberman, A.M., and Christian, M.S. (1985) Detection of prenatal effects on learning as a function of differential criteria. *Neurobehavioral Toxicology and Teratology* 7:697-701. [incorrectly identified as Appendix O, Section 20, pp. 1328-1333]

The submitted information consists of a published article, from 1985, with different authors from the current study, although it was performed at Argus Research Laboratories. In the study, 20-day old pups were tested in a water maze that appears similar to that used in the current study. Several different learning criteria were evaluated, and it was determined that sensitivity of the test procedure for detecting changes in behavior following treatment with test substance depended on the specific learning criteria used (i.e. how many consecutive correct trials were required).

The age at testing was different from that used in the current study, and the procedure appears to have been slightly different. In particular, the authors were not able to demonstrate sensitivity of the test to prenatal alcohol exposure using a learning criteria of 5 consecutive correct trials (the criteria used in the current study). This study is not sufficient to document sensitivity of the procedure as performed in for the current study, and does raise concern about the sensitivity of the current procedures to detect test-material related effects.

Neuropathology, day 12 and adult

12. Neuropathology validation section was provided by Dr. Robert Garman, of Consultants in Veterinary Pathology, who performed the neuropathological evaluations in the submitted study. [incorrectly identified as Appendix O, Section 21, pp. 1334-1391]

43

Garman, R.H. (1996) Neuropathology validation report. Consultants in Veterinary Pathology, P.O. Box 68, Murrysville, PA 15668, Unpublished. -- Validation included a brief description of the consulting neuropathologist's credentials, experience, and publications.

Garman, R.H. (1993) Neuropathology validation report. Consultants in Veterinary Pathology, P.O. Box 68, Murrysville, PA 15668, Unpublished. -- Validation included a brief description of the consulting neuropathologist's credentials, experience, and publications, and presented illustrated descriptions of a variety of neuropathological lesions of the central and peripheral nervous systems in adult rats.

Garman R.H. (1996) Neurotoxicity evaluation of positive control substances in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-058. Unpublished. -- This study used motor activity assessment, auditory startle habituation, and neurohistological examination to evaluate the positive control substances acrylamide, trimethyltin chloride, or MK-801. Motor activity assessment was conducted using similar equipment to that used in the current study; however, sessions were 1.5 hours in duration and comprised of 5 minute blocks, while the current study used 1-hour sessions comprised of 10-minute blocks. Auditory startle habituation testing was conducted using similar equipment and methods as those used in the current study. Similar processing and staining methods were used, and the positive control study evaluated the same brain sections for neuropathology as those evaluated in the F_1 adults in the current study. The validation study for adult neuropathology was missing the data tables, which show the number and type of lesions detected in evaluated rats (these data were listed in the report table of contents as starting on p. 34, but the submitted report ended on p. 33). Since these data are critical in documenting the sensitivity of the study procedures, the results of the study could not be fully evaluated. No validation was included for qualitative neuropathological evaluation of neonatal (day 12) rats, and no neuropathological alterations (or functional effects) were detected in adult rats following prenatal exposure to lead nitrate.

Garman, R.H. (1998) Morphometric measurement validation study comparing day 10 and day 12 pups. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Unpublished. -- This study compared 9 different morphometric measurements between 10 and 12 day old pups. The brains were measured grossly and sectioned similarly to those of the PND 12 pups used in the current study. It was concluded that increases in the thickness of the frontal cortex, height of the cerebellar cortex, and cross-sectional width of the caudate-putamen correlated best with brain maturation between PND 10 and 12. Only the previous two of these three measurements were used in the current study, which also included measurement of the dentate gyrus of the hippocampus. The validation comparing day 10 and day 12 neonatal rats for morphometric measurements was performed on control rats only, and did not demonstrate ability to detect changes following neurotoxic insult. It did, however, demonstrate the ability of the laboratory to detect changes in morphometric measurements from day 10 to day 12.

44

Foss, J., A. Hoberman, and M. Christian (1992) Developmental neurotoxicity evaluation of lead nitrate in in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Horsham, PA. Poster presented at the Annual Meeting of the Society of Toxicology; Seattle, Washington; February 1992. -- The equipment and methods used for motor activity, auditory startle habituation, passive avoidance, and water maze testing were similar to those used in the current study; however no effects of treatment were detected. The neuropathology assessment was performed by Dr. Robert Garman.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range ppm	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
121903	45232501	DNT	Rats	Maternal: GD6-LD10	Oral	Dietary	0, 20, 100, 500	Gestation:: 0, 1.7, 8.3, 40.8 Lactation: 0, 3.0, 15.4, 76.7	NOAEL = 40.8	LOAEL = Not determined		Maternal Transient, minimal decreases in BW, BWG, FC during early gestation were not considered adverse
121903	45232501	DNT	Rats	Maternal: GD6-LD10	Oral	Dietary	0, 20, 100, 500	Gestation:: 0, 1.7, 8.3, 40.8 Lactation: 0, 3.0, 15.4, 76.7	Not determined	LOAEL = 1.7	Decreased BW, BWG	Offspring
									NOAEL = 1.7	LOAEL = 8.3	Delayed eye opening, delayed preputial separation; decreased motor activity for PND 14 females	
									Not determined	LOAEL = 8.3	Decreased caudate putamen measurements for adult female offspring	